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THE EFFECT OF PRESERVATIVES ON FERMENTATION BY SUGAR-TOLERANT YEASTS FROM HONEY[1]

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Abstract

A study was made of the preservative effect of various compounds in an 80% honey solution inoculated with a mixed culture of honey-fermenting yeasts. Sodium hypochlorite and chloramine-T preparations did not prevent fermentation in the highest concentration used (100 p.p.m. available chlorine). Hydrogen peroxide, though effective in a 0.5% concentration, exerted a marked bleaching action in addition to affecting the taste. Hexyl resorcinol completely inhibited fermentation in 0.01% concentration. Complete preservation was not attained with sodium salicylate within the limits permissible in foodstuffs, 0.06% being required. The sodium salts of boric, benzoic and sulphurous acid tested, all prevented fermentation within the limits. Sodium benzoate, sodium sulphite and sodium bisulphite were considered the most promising, and appeared to merit further testing on a practical scale for the prevention of honey fermentation.

Introduction

As a result of a number of investigations (5, 9, 10, 11), spoilage of honey by fermentation is now generally recognized to be due to the action of sugar-tolerant yeasts, capable of fermenting saccharine solutions of high osmotic pressure, which are able to restrain the growth and action of "ordinary" non-osmophilic yeasts such as *Saccharomyces cerevisiae*, etc. Infection of extracted honey by sugar-tolerant yeasts, to some degree at least, may be taken for granted. Although some control of this infection may be effected through the exercise of strictly sanitary precautions at the time of extraction, as pointed out by Lochhead and Heron (9), yet these authors, examining hive nectar throughout the season, found it to be constantly infected. The view that normal honeys regularly contain sugar-tolerant yeasts capable of setting up fermentation under favorable conditions is further strengthened by findings, as yet unreported, resulting from the examination of 200 samples of normal honey from all parts of Canada.

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Apart from storage at low temperature, the means so far recommended to prevent fermentation in stored honey has been the application of heat. In 1910 Nussbaumer (11), testing the effect of heat on a yeast isolated from fermented honey, concluded that heating to 70° C. (158° F.) for 30 min. prevented fermentation. According to Marvin (10) heating honey to 160° F. (apparently without holding at that temperature) was sufficient to kill honey-fermenting yeasts, while Fabian and Quinet (5) report that honey heated to 145° F. for 30 min. is safe from spoilage. There are, however, certain objections to the heating of honey, particularly that for export, inasmuch as certain countries demand unheated honey. A recent enactment in Germany, for instance, classifies all honeys as adulterated in which the diastase content has been destroyed or impaired. Heating honeys to the temperatures mentioned would noticeably affect the diastase content, which even in normal unheated honeys may be low, as shown by Vansell and Freeborn (14).

The effect of preservatives on honey-fermenting yeasts has not received attention, as far as we are aware. Various studies dealing with the action of antiseptics upon living yeast cells and upon the fermentative activity of yeast-juice has been recorded by Harden (7), Guilliermond (6) and Henneberg (8). Much of the previous work has been directed towards discovering antiseptics suitable for use in suppressing undesirable organisms such as lactic acid and acetic acid bacteria, wild yeasts, etc., which affect the desired fermentation by the cultivated yeasts in brewing, wine-making and other industries. Under such circumstances the ideal antiseptic represents one with the greatest inhibiting effect on the contaminating organisms with the least influence on the cultivated yeast. The direct action of various antiseptics and poisons on the growth and fermentation of *Saccharomyces cerevisiae* has also received attention. Thus Perry and Beal (12) found formaldehyde and sodium bisulphite to exert a greater inhibitive effect on fermentation than sodium benzoate, sodium sulphite, sodium salicylate or alcohol. Brown and Wikoff (2) found hydrogen peroxide to exert a noticeably inhibiting effect on fermentation, while the same authors (3) found hexyl resorcinol to be strongly toxic for yeasts. Dann and Quastel (4) in studying the effects of certain glucosides and polyhydric phenols on fermentation, found allyl alcohol and acrylic acid to exert a retarding action on fermentation of glucose by live yeast and by zymin. Phloridzin showed an inhibitive effect on zymin fermentation of glucose though not with live yeast. Branham (1), investigating the effects of various chemicals on gas production by baker's yeast, determined the concentrations at which fermentation was completely inhibited. In many cases an apparent stimulation of CO_2 production was observed with low concentrations of certain compounds which, in higher concentrations, were able to prevent CO_2 formation.

Experimental Work

In selecting the preservatives used in the experiments here reported, consideration was given to those of a non-poisonous nature, that is, with a low

degree of toxicity for animal organisms, and particularly those the use of which is, or might be, permitted in foodstuffs. Accordingly the following substances were chosen, the concentrations employed being selected to include the limits permissible under the regulations of the Food and Drugs Act where given therein.

1. Sodium benzoate: 0.01, 0.025, 0.05, 0.075, 0.10, 0.125, 0.15 and 0.2%, calculated as benzoic acid. Limit, 0.10%.

2. Sodium borate (borax): 0.05, 0.10, 0.25, 0.50, 0.75 and 1.0%, calculated as B_2O_3. Limit, 0.50%.

3. Sodium salicylate: 0.0025, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08 and 0.10%, calculated as salicylic acid. Limit, 0.02%.

4. Sodium sulphite (anhydrous): 0.01, 0.025, 0.035, 0.05, 0.075, 0.10, 0.15 and 0.20%, calculated as SO_2. Limit, 0.05%.

5. Sodium bisulphite: 0.01, 0.025, 0.035, 0.05, 0.075, 0.10, 0.15 and 0.20%, calculated as SO_2. Limit, 0.05%.

6. "B-K" (sodium hypochlorite): 10, 25, 35, 50, 75 and 100 parts per million available chlorine.

7. "Santomine" (chloramine-T): 10, 25, 35, 50, 75 and 100 parts per million available chlorine.

8. Hexyl resorcinol: 0.001, 0.002, 0.005, 0.01, 0.015 and 0.02%.

9. Hydrogen peroxide (superoxyl): 0.01, 0.05, 0.10, 0.20, 0.50, 1.0 and 1.5% H_2O_2.

Due to the comparative slowness with which fermentation in pure honey makes itself manifest, it was decided to use, for this series, 80% (by weight) honey solution in order to encourage the action of the honey-fermenting yeasts and thus permit differences to be apparent within a reasonable period of time. Tests were conducted in 300 cc. Erlenmeyer flasks fitted with Alwood fermentation valves, 200 gm. of the honey solution* being measured into each flask. The preservatives were added in concentrated aqueous solution and well mixed with the honey before making up to final weight. In the case of hexyl resorcinol, which is practically insoluble in water, a solution in alcohol and glycerol was prepared. For each concentration of the preservative tested, duplicate flasks were inoculated with equal amounts of a mixed culture of four sugar-tolerant yeasts (cultures J7, E6, D1 and M1) isolated from samples of fermented honey (9). Each set included, in addition, two control flasks containing no preservative. All flasks were incubated at 28° C. for a month, observations made of fermentation, and loss in CO_2 measured by weighing every four or five days.

* *The honey was not sterilized for use, the tests being considered more valuable from a practical standpoint when conducted on honey not so treated.*

Discussion of Results

From Table I, in which is presented a summary of the results given detail in Table II to IV, and from Fig. 1 to 8, showing average losses in CO_2 from the duplicate flasks, considerable variation will be noted in the inhibiting action of the different materials examined.

Both of the chlorine compounds tested, B-K (Table III) and Santamine (Table IV), (see Fig. 5 and 6) were unsatisfactory, the highest concentrations in each case, namely 100 p.p.m. of available chlorine, failing to halt fermentation of the honey solution. In addition, the marked chlorine flavor in all samples, which persisted until the end of the experiment, sufficed to make their employment impracticable.

Hydrogen peroxide likewise proved unsuitable under the conditions of the test. Although a concentration of 0.5% H_2O_2 inhibited fermentation (Fig. 8, Table IV), yet in this strength there was a marked taste. Furthermore, a noticeable bleaching of the honey took place, particularly with the higher concentrations of preservative.

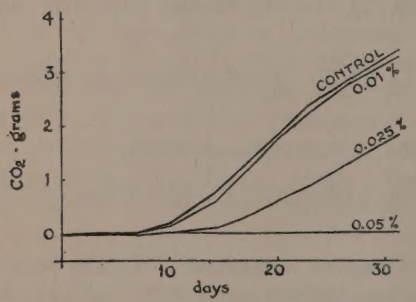

FIG. 1. *Effect of sodium benzoate on fermentation.*

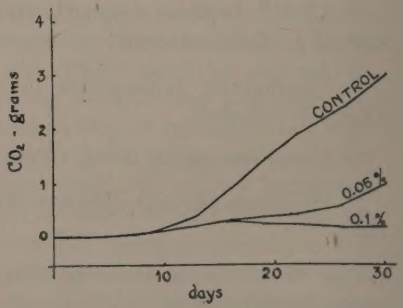

FIG. 2. *Effect of sodium borate on fermentation.*

Hexyl resorcinol, a comparatively recent synthetic antiseptic, is noted for its high bactericidal action (phenol coefficient 45-55), while possessing at the same time a proportionately low toxicity to animal organisms (2). This compound was found to exert a decidedly inhibiting effect on the action of honey-fermenting yeasts, a concentration of 0.01% being sufficient to prevent fermentation (Fig. 7 and Plate I). A like concentration was found by Brown and Wikoff (2) to inhibit growth and fermentation by baker's yeast. Although in the honey tests the action of hexyl resorcinol was promising yet its insolubility in water and its relatively high cost tend to lessen its practical usefulness.

More promising results were obtained with the sodium salts of benzoic, boric and sulphurous acid. These were all found to exert, under the experimental conditions, a preventive effect on fermentation within the limits

TABLE I

EFFECT OF PRESERVATIVES ON HONEY FERMENTATION (80% HONEY)

Preservative	Limit permissible	Highest concentration, fermenting	Lowest concentration not fermenting	Effect on flavor	Remarks
Sodium benzoate	0.10% as benzoic acid	0.025%	0.05%	not detectable	promising
Sodium borate	0.5% B_2O_3	0.05%	0.1%	not detectable up to 0.5%, salty above	low solubility a disadvantage, otherwise promising
Sodium salicylate	0.02% as salicylic acid	0.04%	0.06%	not detectable	no preservation within limits permissible
Sodium sulphite	0.05% SO_2	none fermented, 0.01% lowest concentration used.	0.01%	seemed to bring out honey flavor	promising
Sodium bisulphite	0.05% SO_2	0.1%	0.025%	seemed to bring out honey flavor	promising
B-K (sodium hypochlorite)		all fermented, 100 p.p.m. Cl_2 highest concentration used		marked chlorine flavor in all	unsuitable
Santamine (chloramine-T)		all fermented, 100 p.p.m. Cl_2 highest concentration used		marked chlorine flavor in all	unsuitable
Hexyl resorcinol		0.005%	0.01%	not detectable	insolubility in water and cost are disadvantages
Hydrogen peroxide		0.2%	0.5%	quite marked flavor in concentrations inhibiting fermentation	unsuitable on account of taste and bleaching effect

TABLE II

EFFECT OF PRESERVATIVES ON LOSS IN WEIGHT IN HONEY SOLUTION INOCULATED WITH HONEY-FERMENTING YEASTS

Preservative	Loss in weight (gm.)								Fermented	
									Yes	No
Sodium benzoate	Days *4*	*10*	*14*	*17*	*20*	*23*	*27*	*31*	+	
control	0.05	0.25	0.8	1.35	1.85	2.4	2.9	3.4	+	
0.01 %	0.05	0.2	0.6	1.2	1.8	2.25	2.8	3.3	+	
0.025%	0.0	0.05	0.15	0.35	0.65	0.9	1.35	1.85	+	
0.05 %	0.05	0.05	0.05	0.05	0.15	0.1	0.1	0.1		+
0.075%	0.0	0.05	0.1	0.15	0.2	0.15	0.05	0.1		+
0.1 %	0.05	*0.15*	*0.05*	0.0	0.0	*0.05*	*0.1*	*0.1*		+
0.125%	0.0	0.1	0.1	0.2	0.2	0.2	0.1	0.0		+
0.15 %	0.0	*0.1*	*0.1*	0.0	0.05	0.05	*0.1*	0.0		+
0.2 %	0.0	0.1	0.0	0.15	0.15	0.1	0.05	0.1		+
Sodium borate	Days *3*	*9*	*13*	*16*	*19*	*22*	*26*	*30*		
control	0.0	0.05	0.4	0.9	1.45	1.9	2.35	2.95	+	
0.05 %	0.1	0.1	0.2	0.3	0.35	0.4	0.55	0.95	+	
0.1 %	0.15	0.15	0.15	0.3	0.25	0.25	0.15	0.25		+
0.25 %	0.15	0.2	0.25	0.3	0.45	0.25	0.2	0.25		+
0.5 %	0.05	0.1	0.1	0.2	0.25	0.2	0.1	0.2		+
0.75 %	0.15	0.1	0.15	0.2	0.25	0.2	0.1	0.15		+
1.0 %	0.3	0.3	0.1	0.1	0.1	0.1	0.2	0.1		+
Sodium salicylate	Days *4*	*7*	*11*	*17*	*21*	*24*	*29*	*32*		
control	0.05	0.1	0.1	0.9	1.55	1.95	2.75	3.0	+	
0.0025%	0.1	0.1	0.25	0.95	1.6	2.15	2.95	3.25	+	
0.005 %	0.1	0.1	0.05	0.9	1.5	1.95	2.7	2.95	+	
0.01 %	0.05	0.15	0.1	0.6	1.3	1.7	2.5	2.85	+	
0.02 %	0.05	0.1	0.15	0.0	0.1	0.45	0.9	1.2	+	
0.04 %	0.1	0.1	0.2	0.1	0.2	0.2	0.35	0.6	+	
0.06 %	0.05	0.05	0.05	0.05	0.05	0.0	*0.1*	*0.05*		+
0.08 %	0.1	0.05	0.25	0.2	0.1	0.05	*0.05*	0.05		+
0.1 %	0.0	0.1	0.15	0.15	0.1	0.05	*0.05*	0.0		+

NOTE:—*Figures printed in italics indicate gain in weight.*

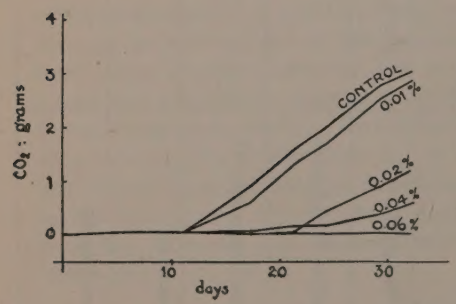

FIG. 3. *Effect of sodium salicylate on fermentation.*

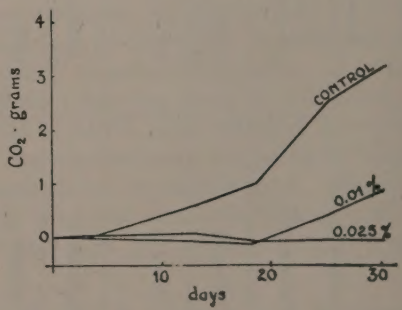

FIG. 4. *Effect of sodium bisulphite on fermentation.*

TABLE III

EFFECT OF PRESERVATIVES ON LOSS IN WEIGHT IN HONEY SOLUTION INOCULATED WITH HONEY-FERMENTING YEASTS

Preservative	Loss in weight (gm.)								Fermented	
									Yes	No
Sodium sulphite	Days *5*	*9*	*12*			*23*	*27*	*30*		
control	0.15	0.3	0.4			0.75	1.25	1.7	+	
0.01 %	0.2	0.35	0.3			0.25	0.4	0.35		+
0.025 %	0.2	0.4	0.35			0.3	0.45	0.4		+
0.035 %	0.15	0.35	0.35			0.25	0.5	0.4		+
0.05 %	0.15	0.35	0.3			0.3	0.5	0.35		+
0.075 %	0.15	0.35	0.35			0.25	0.35	0.35		+
0.1 %	0.15	0.35	0.35			0.3	0.4	0.4		+
0.15 %	0.15	0.4	0.25			0.35	0.1	0.3		+
0.2 %	0.1	0.3	0.3			0.3	0.4	0.4		+
Sodium bisulphite	Days *3*		*13*	*18*		*25*		*30*		
control	0.0		0.6	1.0		2.55		3.15	+	
0.01 %	0.0		*0.05*	*0.15*		0.45		0.9	+	
0.025 %	0.05		0.2	*0.1*		0.0		0.0		+
0.035 %	*0.15*		0.05	*0.1*		0.1		*0.25*		+
0.05 %	0.1		0.3	0.05		0.2		*0.05*		+
0.075 %	*0.2*		0.15	*0.35*		*0.05*		*0.25*		+
0.1 %	*0.1*		0.1	*0.15*		0.05		*0.2*		+
0.15 %	*0.1*		0.2	0.25		0.2		0.0		+
0.2 %	*0.1*		0.15	*0.1*		0.15		0.0		+
"B-K" (Sodium hypochlorite)	Days *5*	*10*	*15*	*19*	*21*	*26*	*29*	*31*		
control	0.1	0.35	1.0	2.0	2.2	2.95	3.3	3.55	+	
10 p.p.m. Cl	*0.15*	*0.05*	0.55	1.4	1.75	2.45	2.9	3.15	+	
25 p.p.m. Cl	*0.1*	0.2	0.95	2.1	2.25	3.0	3.35	3.55	+	
35 p.p.m.	*0.2*	0.05	0.55	1.65	1.7	2.7	3.15	3.35	+	
50 p.p.m.	*0.15*	0.05	0.45	1.4	1.65	2.45	2.95	3.4	+	
75 p.p.m.	*0.15*	0.0	0.55	0.9	1.0	1.8	2.1	2.45	+	
100 p.p.m.	*0.1*	0.0	0.2	0.85	0.85	1.7	2.1	2.5	+	

NOTE:—*Figures printed in italics indicate gain in weight.*

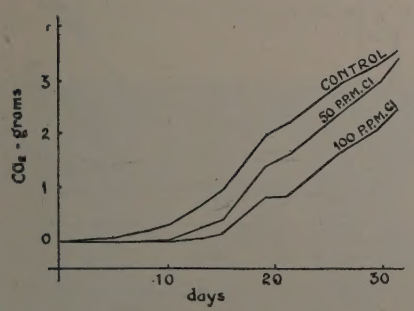

FIG. 5. *Effect of "B-K" (Sodium hypochlorite) on fermentation.*

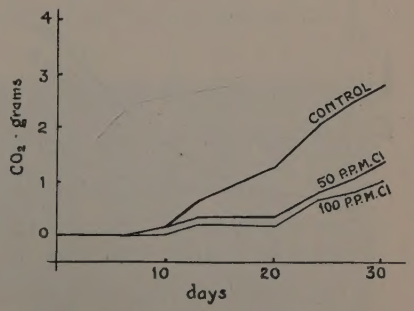

FIG. 6. *Effect of "Santamine" (Chloramine-T) on fermentation.*

TABLE IV

EFFECT OF PRESERVATIVES ON LOSS IN WEIGHT IN HONEY SOLUTION INOCULATED WITH HONEY-FERMENTING YEASTS

Preservative	Loss in weight (gm.)								Fermented	
									Yes	No
"Santamine" (Chloramine-T)	Days 6	*10*	*13*		*20*	*24*	*27*	*30*		
control	0.05	0.3	0.3		0.5	0.75	1.75	2.15	+	
10 p.p.m.Cl	0.0	0.2	0.2		0.2	0.65	0.95	1.35	+	
25 p.p.m.	0.0	0.15	0.2		0.35	0.95	1.3	1.75	+	
35 p.p.m.	0.05	0.1	0.25		0.1	0.05	0.65	0.95	+	
50 p.p.m.	0.1	0.2	0.4		0.35	0.85	1.05	1.4	+	
75 p.p.m.	0.0	0.1	0.3		0.05	0.25	0.25	0.35	+	
100 p.p.m.	0.0	0.1	0.25		0.15	0.7	0.8	1.1	+	
Hexyl resorcinol	Days 5	*10*	*15*		*19*	*22*	*27*	*30*		
control	0.05	0.15	0.8		1.3	2.0	2.85	3.25	+	
0.001%	0.05	0.05	0.7		1.35	1.95	2.8	3.2	+	
0.002%	0.05	0.3	0.65		1.0	1.65	2.3	2.65	+	
0.005%	0.05	0.2	0.2		0.2	0.5	0.85	1.05	+	
0.01 %	0.0	0.1	0.05		*0.05*	0.0	0.0	*0.05*		+
0.015%	0.05	0.15	0.15		0.0	0.0	0.05	*0.05*		+
0.02 %	0.0	0.1	0.2		*0.05*	0.05	0.05	*0.05*		+
Hydrogen peroxide	Days 3	*9*	*13*	*16*	*20*	*23*	*27*	*31*		
control	0.0	0.25	0.75	1.0	1.85	2.5	3.25	3.9	+	
0.01 %	0.05	0.2	0.6	0.9	1.75	2.4	3.15	3.7	+	
0.05 %	0.05	0.2	0.55	0.65	1.35	1.95	2.5	3.35	+	
0.10 %	0.05	0.15	0.3	0.2	0.5	0.9	1.65	2.45	+	
0.2 %	0.1	0.3	0.4	0.3	0.25	0.3	0.55	0.7	+	
0.5 %	0.15	0.3	0.35	0.15	0.2	0.1	0.2	0.1		+
1.0 %	0.15	0.3	0.4	0.2	0.2	0.25	0.25	0.25		+
1.5 %	0.0	0.1	0.2	0.05	0.0	0.05	0.05	0.1		+

NOTE:—*Figures printed in italics indicate gain in weight.*

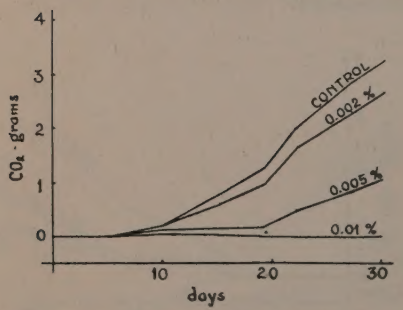

FIG. 7. *Effect of hexyl resorcinol on fermentation.*

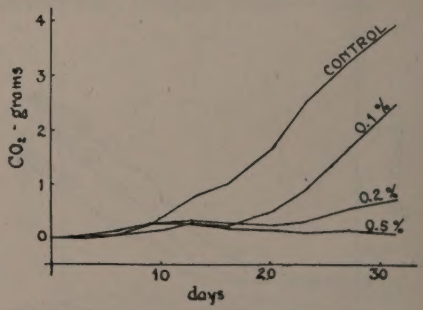

FIG. 8. *Effect of hydrogen peroxide on fermentation.*

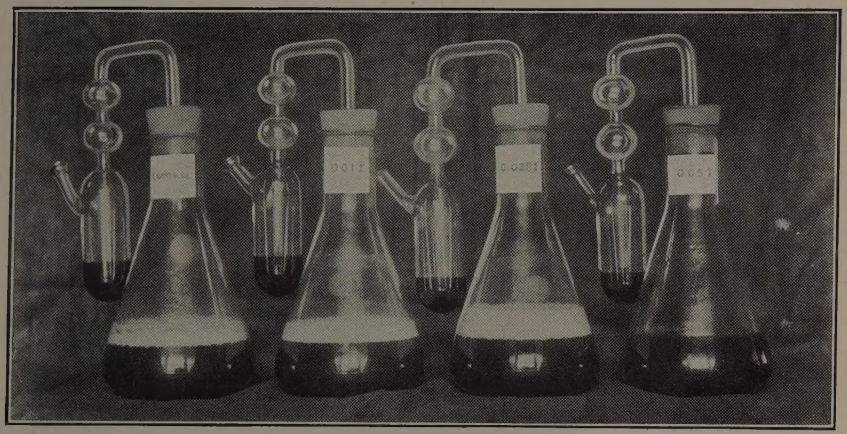

FIG. 1. *Effect of sodium benzoate on fermentation of 80% honey solution.*

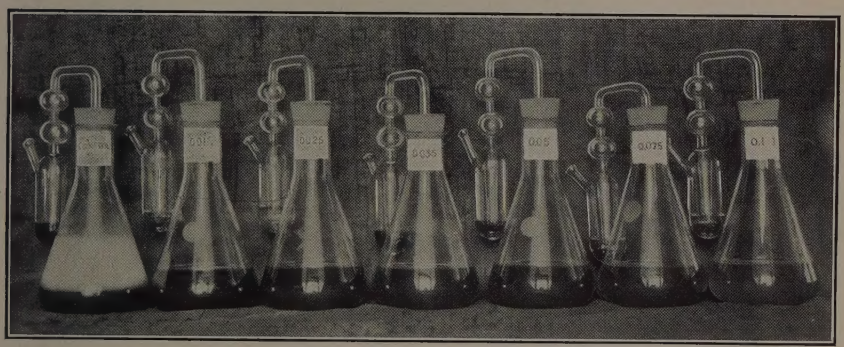

FIG. 2. *Effect of sodium sulphite on fermentation of 80% honey solution.*

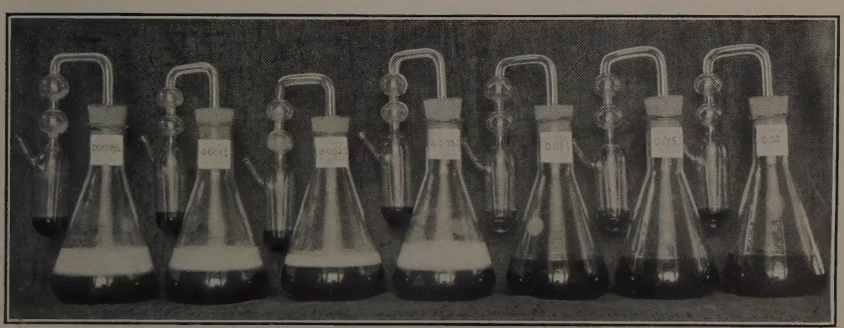

FIG. 3. *Effect of hexyl resorcinol on fermentation of 80% honey solution.*

permissible in foodstuffs (Fig. 1-2, Table II and Plate I). Sodium salicylate, on the other hand, was found incapable of exerting an inhibiting effect within the limits, a concentration of 0.06% being required to halt fermentation.

Sodium borate, though successful in preventing fermentation within the limits allowed, requires not only a heavier concentration for this inhibiting effect, but has the disadvantage of low solubility in water. The compounds, therefore, which appear to merit further testing on a practical scale are sodium benzoate, sodium sulphite and sodium bisulphite.

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THE EFFECT OF THE ULTRA-VIOLET COMPONENT OF SUNLIGHT ON CERTAIN AQUATIC ORGANISMS[1]

By A. Brooker Klugh[2]

Abstract

In these experiments, which are a continuation of work carried out in 1927 and 1928, certain aquatic animals were exposed to sunlight behind filters which transmitted different regions of the solar spectrum. The first filter transmitted both the visible and ultra-violet regions, the second the visible only and the third the ultra-violet only. Young eels, *Anguilla rostrata*, which are in shallow water when ascending the streams, an Amphipod, *Gammarus locusta*, which was taken from shallow tide-pools, and a Ctenophore, *Bolinopsis infundibulum*, which floats about at the surface of the sea, all proved to be very resistant to ultra-violet radiation, thus showing a marked contrast to those animals which remain at some considerable depth in the sea or which come to the surface only when the illumination is very weak, as the latter are killed in a short time by exposure to ultra-violet radiation.

The data presented in this brief paper are the result of the continuation of a series of experiments on the effect of the sun's ultra-violet radiation on organisms, which were carried on in 1927 and 1928 at the Atlantic Biological Station, St. Andrews, N.B. The present paper deals with the work done during the summer of 1929.

The apparatus and the method of experimentation were the same as those employed in 1927 (1, 2), except that, as none of the animals used in these experiments required a water temperature lower than 15° C., the water which flowed through the filter-box was not brought through the copper coil immersed in a mixture of ice and salt. In these experiments the organisms were placed in each compartment of the filter-box, and exposed to sunlight passed through the various filters transmitting different regions of the solar spectrum.

Experiments on young Anguilla rostrata

July 10, 1929. 3.00 p.m. One young eel, *Anguilla rostrata*, placed in each compartment. Sun, with cumulus clouds all day.

7.00 p.m. All normal.

July 11, 9.00 a.m. Under Novial filter (a filter which transmitted the whole of the visible spectrum but no ultra-violet)—Normal.

Under Total filter (a filter which transmitted both visible and ultra-violet radiation)—Weak.

Under Ultra-violet filter—Dead.

A new animal placed in the ultra-violet compartment.

Bright sun with some cirrus clouds all day. Pyrheliometer (Py.) reading (max.) 1.26 gm. cal. per sq. cm. per min.

4.00 p.m. Under Novial—Normal.

Under Total—Weak.

Under Ultra-violet—Normal.

[1] *Manuscript received May 28, 1930.*

Contribution from the laboratories of Queen's University, Kingston, Ontario, with financial assistance from the National Research Council of Canada, and the co-operation of the Biological Board of Canada.

[2] *Associate Professor of Biology, Queen's University, Kingston, Ontario.*

July 12, 9.00 a.m. Under Novial—Normal.
Under Total—Weak.
Under Ultra-violet—Dead.
New animal placed under Ultra-violet.
Bright sun with cirrus and cumulus clouds all day.
6.30 a.m. Under Novial—Normal.
Under Total—Dead.
Under Ultra-violet—Dead.

Experiments on Gammarus locusta

July 17, 9.30 a.m. Two amphipods (*Gammarus locusta*) placed in each compartment. Bright sun with cumulus clouds all day.
7.00 p.m. All normal.

July 18, 9.00 a.m. All normal.
Bright sun with cumulus clouds all day. Py. 1.21.
7.00 p.m. All normal.

July 19, 9.00 a.m. All normal.
Fog and rain all day.

July 20, 1.00 p.m. Novial—Normal and five young had been produced.
Total—Normal.
Ultra-violet—Both dead.
Placed one young under Total and one under Ultra-violet.
Bright sun with cumulus clouds all day.

July 21, 2.00 p.m. All normal.
Bright sun with cirrus clouds all day. Py. 1.19.

July 22, 11.00 a.m. All normal.
Bright sun with cirrus clouds all day.

July 23, 9.00 a.m. Novial—Normal.
Total—Normal.
Ultra-violet—Dead.
Placed another young in Ultra-violet.
Bright sun with cirrus clouds all day.

July 24, 9.00 a.m. Novial—Normal.
Total—Adults normal, young dead.
Ultra-violet—Young put in yesterday still alive.

Experiments on Bolinopsis infundibulum

August 1, 10.00 a.m. One *Bolinopsis infundibulum*, a Ctenophore which floats about near the surface of the sea, placed in each compartment.
6.00 p.m. All normal.

August 2, 9.00 a.m. All normal.
6.00 p.m. All normal.

August 3, 9.00 a.m. All dead.

August 2, 9.00 a.m. One young *B. infundibulum* placed in each compartment.
6.00 p.m. Novial—Normal.
Total—Dead.
Ultra-violet—Normal.

August 3, 9.00 a.m. All dead.

August 9, 9.00 a.m. One *B. infundibulum* placed in each compartment.
6.00 p.m. Novial—Normal.
Total—Weak.
Ultra-violet—Weak.

August 10, 12.00 noon. All dead.

Discussion

These experiments bear out the conclusions reached in the two preceding papers on this subject,—that animals which live at some considerable depth in the sea, or which come to the surface only when the illumination is very weak, are readily killed by exposure to ultra-violet radiation, but are not affected by visible radiation, while those which live at or near the surface of the sea, or in shallow fresh water, can be killed only by much more prolonged exposure to ultra-violet. Thus the young eels, which are in shallow water when ascending the streams, proved to have considerable resistance to ultra-violet radiation. The same is true for *Gammarus locusta*, the specimens of which were taken from a shallow tide-pool. *Bolinopsis infundibulum* which floats at the surface of the sea showed so little difference in its resistance under ultra-violet and visible radiation that death was probably due to factors other than a difference in the wave-length of the radiation to which it was exposed.

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SINKAGE STUDIES IV. THE MECHANISM OF THE ABSORPTION OF WATER BY WOOD BLOCKS[1]

By Geo. W. Scarth[2]

Abstract

Uniform blocks of white pine wood were allowed to absorb water under various conditions of external and internal pressure, and in the presence and absence of air. The results shown graphically give some idea of the quantitative relation existing between penetration of water into wood and the various forces operating. It is concluded that the rate of penetration of water into an immersed block of coniferous heartwood is fixed by the rate at which the air in the block can dissolve and diffuse out.

Applied to log sinkage, these findings show that under most conditions the limiting factor is the rate of escape of air from the log.

In the preceding paper (2) it was stated that the essential process in sinkage is the solution of enveloped gas bubbles and their replacement by water, though this may be complicated by evaporation and the currents which it induces, and also by fermentative evolution of gas inside the log. The purpose in this paper is to exclude these complications and obtain quantitative data as to the relation between the two fundamental processes and the forces involved, with a view to intelligent control of the sinkage process.

Blocks of coniferous (white pine) heartwood were used on account of its low permeability and lack of fermentable foodstuff.

Let it first be considered from the theoretical standpoint what the factors are that determine the rate at which water will be absorbed by a block of wood. Starting with wood in the dry state, water enters both as vapor and as liquid. The result of distillation of water vapor into the cavities of the wood is to saturate the cell walls long before *free* water has penetrated to any great distance. (This was seen in the flotation of dry logs and will appear again in the experiments which follow.) Vaporous diffusion across the cavities is accompanied by movement of imbibed water through the cell walls, especially as regards transmission of moisture from one cell to the next. The progress of imbibition can be followed by measuring volume, since the relation between swelling and moisture content is a linear one (Goss, see Hawley and Wise) (1).

Simultaneously with distillation and imbibition there is a flow of free liquid water into the block, and, once complete saturation of the enclosed air and the cell walls has put an end to the former process, all the water is absorbed in the free and liquid form. Most attention, therefore, must be directed to the factors which govern the rate of *liquid penetration.*

Like any other current, flow of water into wood varies directly as the driving pressure and inversely as the frictional resistance:

$$C \propto \frac{P}{R}$$

R, the frictional resistance, depends on the structure and density of the wood

[1] *Manuscript received May 28, 1930.*
Contribution from the Department of Botany, McGill University, Montreal.
[2] *Professor of Botany, McGill University.*

and increases with the main distance the water has to travel. P, (near the outside of the block, at least) equals atmospheric pressure+hydrostatic pressure+capillary pressure−pressure of the air in the block. A diagram of the conditions in a block and of the forces at play is given in Fig. 1.

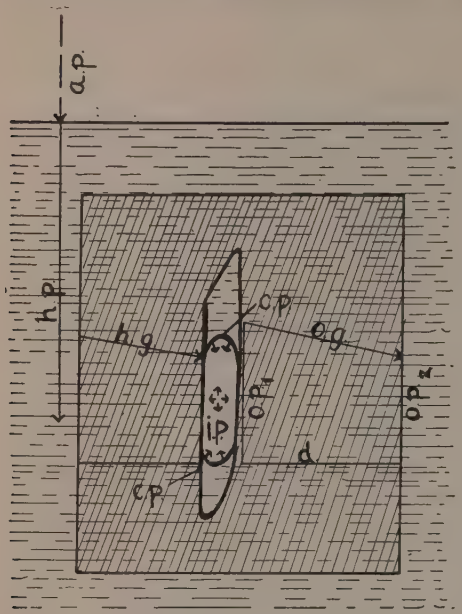

FIG. 1. *Diagram of absorption of water and escape of air in an immersed wood block: the forces at play.*

a. p. atmospheric pressure
h. p. pressure of the water
h. g. (slope) hydrodynamic gradient
c. p. capillary pressure
i. p. internal air pressure.
o. p. osmotic pressure by dissolved air
o. g. (slope) osmotic gradient, equal to $\frac{op_1 - op_2}{d}$

Since the condition is hydrokinetic, there will be a certain falling off in pressure from the outside inward, due to the increasing resistance. As regards the opposing air pressure, it may be noted that in the experiments which follow (as in the earlier experiments with logs) scarcely any air escapes as bubbles. It is enveloped by water and enclosed in the cell cavities. Its pressure at the start is normally atmospheric. Whether the pressure in the bubbles rises above atmospheric pressure or falls below it during absorption depends on the relative speeds of access of water and escape of air. If the water enters the faster, it compresses the air. If the air dissolves the faster, it creates a partial vacuum. The rate of solution, in turn, depends on the rate at which the dissolved air can diffuse away. This outward diffusion current, like the inward current of water, varies as the driving pressure and inversely as the friction:

$$C \propto \frac{p}{r}$$

r, as before, depends largely on the structure and density of the wood, but the water itself also offers resistance to diffusion. p is the gradient osmotic pressure of dissolved gas between the interior and outside. It decreases in proportion to the distance. Of course, these two processes—inflow of water and outward diffusion of air—are interdependent. Increased pressure in the air bubbles both resists infiltration and promotes exo-diffusion: decreased pressure has the opposite effect. Yet the term "limiting factor" may be applied to that which lags behind the other.

The problem is (a) to find out which is the limiting factor under different conditions and (b) to obtain, if possible, quantitative data as to relation between rate of absorption and the various pressures and resistances enumerated above. Such information should serve as a guide to the most effective methods of control.

The general method of analysis which has been used consists in measuring and comparing rates of absorption when various factors are modified separately. In the present series of experiments, the pressure relations and osmotic gradients are variously modified while the wood is unchanged. Experiments are in progress, and will be completed shortly, in which the resistance only is varied, determining the penetration along different axes and on different species of wood under ordinary conditions of pressure.

Description of Experiments

The blocks measured about 9 cm. along the longitudinal axis, 2.8 cm. along the tangential and 0.8 cm. along the radial direction. They were unpainted, so that radial penetration predominated as in absorption by logs. The pieces were cut end to end and exposed simultaneously to the different modes of treatment; the same piece was also exposed successively to the different modes of treatment. Variations in permeability due to slight structural differences in the one case and to solution of material from the wood in the other case can both be discounted. In Fig. 2-4, each curve, in most cases, is for a single sample. In those lettered alike the same sample was used, but the different samples were also found to follow much the same curve.

In every experiment the blocks were immersed to a depth of 10 cm. giving a hydrostatic pressure of about 0.01 atmosphere. The pressure over and above this was varied.

The capillary pressure or tension due to the curvature of the menisci may roughly be calculated. The mean internal diameter of most of the tracheids ranges from 30 to 36μ; a few extremes reach 50 and 10μ respectively. In glass tubes a diameter of 30 to 36μ would give a lift of from 0.1 to 0.08 atmosphere. According to Strasburger, however, the capillary rise of water in wood elements is much less — sometimes less than half — than that of pure water in glass tubes. No doubt the surface tension is lowered by surface active substances in the wood. Probably, therefore, a capillary pressure of more than 0.05 atmosphere on an average cannot be expected to be exerted on the enclosed air bubbles. Even so, it is five times the hydrostatic pressure which is operating in the experiments and under ordinary floating conditions. Only at the very start when the pointed ends of the tracheids begin to fill up, at the last stages when the bubbles are of less diameter than the tracheids and at all stages in the late summer wood, will higher capillary pressures be developed. Opposed to these pressures is the pressure of the air or vapor enclosed in the wood; in most of the experiments this was atmospheric at the start but in some cases it was reduced nearly to zero.

The treatments were as follows:—

Blocks dried in the oven

(1) Placed in water saturated with air.
(2) Placed in water freed from air.
(3) Evacuated of air by oil pump and placed in air-free water under vacuum.
(4) Placed in air-free water under two atmospheres pressure (atm.+750 mm. Hg).
(5) Evacuated of air and covered by air-free water under atmospheric pressure.
(6) Placed in air-free water under 2.33 atmospheres pressure (atm.+1,000 mm. Hg).

Blocks air-dried

(1) Placed in water saturated with air.
(2) Placed in air-free water.
(3) Placed in strong NaOH (about 3.5 *N*).

The respective pressures at the outset of absorption were approximately as given in Table I.

TABLE I
INITIAL PRESSURES

Treatment	External pressure	Internal pressure	Difference
1	1.06 atm.	1.02	.04
2	1.06	1.02	.04
3	0.06	0.02+	.04−
4	2.06	1.02	1.04
5	1.06	0.02+	1.04−
6	2.4	1.02	1.4

The second decimal figure is uncertain. In the column "External pressure" this figure stands for capillary pressure+pressure of the water column; in the column "Internal pressure" it stands for the pressure of water vapor in the tracheids. Being the same in each case, a small error in its actual value does not matter. Of course, the atmospheric pressure itself varies considerably but in parallel experiments it operates equally on all. An important error occurs in the internal pressure after vacuum. It is difficult to get rid of adsorbed air and it is certain that some remained and was slowly released during the experiment.

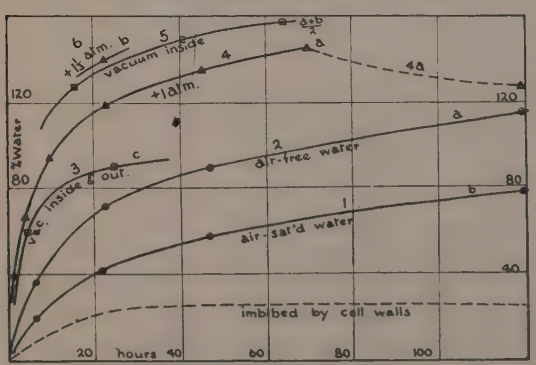

FIG. 2. *The rate of penetration of water into wood under various conditions.*

The results are shown graphically in Fig. 2, the numbers of the curves corresponding to those of the treatments.

In comparing absorption under different conditions it is better, in order to equalize resistance as far as possible, to take the same stage of absorption and compare times, rather than to compare amounts of water absorbed in a given time. When the same depth of penetration is reached, the same resistance by the cell walls has been experienced. The only trouble is that an equal amount of absorption does not in every case indicate an equal depth of penetration. Thus, in air-saturated water, bubbles persist in the outer wood while penetration goes beyond them and reduces the air volume in the interior; in air-free water the air is soon dissolved from the outer tracheids, while farther in, the same procedure takes place as with water originally air-saturated; thirdly, in blocks which have been evacuated of air there is a progressive filling up of the tracheids from the outside inward. In the above order, therefore, there is a decreasing depth of penetration and a decreasing resistance to be overcome with a given amount of absorption.

This makes it very difficult to estimate quantitatively the relation between the various pressures and rate of absorption. A fuller account of the experiments and their results follows.

Results

Variation of osmotic gradient of dissolved air

In the first experiment (Treatments 1 and 2) the pressures are the same at the start but change differently as time goes on. Comparing the curves 1 and 2 in Fig. 2-4, curve 1 shows the absorption of air-saturated and curve 2 of air-free water. The fundamental variant here is the gradient of concentration of dissolved air which affects the rate of diffusion of air outward, and thus indirectly the pressure of air in the block. A considerable part of the absorption in the early stages is water of imbibition. Entering as vapor or along the walls, it is not affected by the presence of dissolved air. An endeavor has, therefore, been made in Fig. 4 to show the "free" water absorbed into the cavities by subtracting the imbibed water, estimated from volume change, from the total. After the first inrush, the air-saturated water takes over five times as long to reach a given stage of absorption as does the air-free water. In the former, at least, the escape of air must limit the rate of penetration, as was already inferred from the preceding experiments. This is also proved by the distribution of air in the wood. Up to the end of the experiment the block in aërated water retained its pearly-whitish appearance, due—as microscopic examination confirmed—to air bubbles in the very outer tracheids. The other block within a few hours after immersion assumed a yellowish translucent aspect and examination at the end of nine days showed that, up to a depth of over 1 mm., air had almost completely disappeared from the wood.

After a time, however, the two absorption curves became parallel and even slightly convergent (Fig. 3) showing that with deeper penetration the resistance factor dominates in both. The question to be solved is whether, in air-free water, it is resistance to escape of air or entry of water.

It is, of course, the capillary and hydrostatic pressure alone which tends to make the air dissolve in water already saturated at atmospheric pressure. In this case the pressure in the bubbles must rise above atmospheric pressure. As regards the condition in air-free water, observation of the air bubbles in the cells, immediately on sectioning, shows that they do not contract in volume and that therefore their pressure has become approximately equal to atmospheric capillary pressure, and must oppose the entry of water. Here also, then, the rate of diffusion of air, in spite of the much higher osmotic gradient, is the lagging factor.

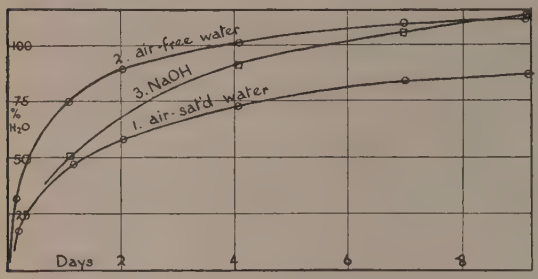

FIG. 3. *Comparative rates of penetration into wood of 1, air-saturated water; 2, air-free water; 3, sodium hydroxide.*

Curve 3 in Fig. 3 shows penetration of strong sodium hydroxide (about 3.5 *N*). The weight increase is corrected for the specific gravity of the sodium hydroxide (1.09), assuming that it penetrates in the same concentration as it has outside. At first the rate resembles that of air-saturated water, but it increases relatively to both the others as time goes on—probably because it alters the permeability of the wood.

Variation of internal air pressure and external pressure

Since even in air-free water the limiting factor in rate of absorption appears to be rate of escape of air, the air was more or less eliminated from the blocks by a Cenco-Hyvac oil pump (28 hr. pumping). The blocks were then covered by air-free water without access of air and in the experiment, the results of which are illustrated by curve 3 in Fig. 2, they continued under the vacuum of an oil or water pump. Thus there was a good vacuum both outside and inside. There was at first much more rapid absorption than when air was present in the block and the pressure of the atmosphere was acting on the outside. This is what would be expected if the air pressure inside, in the latter case, rose above atmospheric pressure, as was indicated by observation of the air bubbles. The flattening out of curve 3 as time goes on is almost certainly due to incomplete evacuation of air. It takes many days under very good vacuum to draw off the adsorbed air.

The disturbing effect of a residual internal atmosphere is reduced by increasing the external pressure. Blocks were therefore evacuated for 20 hr. and then covered by water under atmospheric pressure (curve 5).

The result of a parallel experiment is shown in curve 4. Here the conditions were as in 1, except that an additional atmosphere of pressure (750 mm. Hg) was superimposed externally. In curve 5 the conditions were as in 3,

except that the pressure of the atmosphere was allowed to play on the liquid. The difference between the initial pressures outside and inside was about one atmosphere in both cases. Yet, with a near vacuum inside, the rate of absorption was about twice that with air inside. The air must be strongly compressed in the latter case. This was strikingly demonstrated by the fact that removal of the extra pressure caused exudation of water from the block while still immersed. A block lost about 20% of its dry weight in two days (curve 4a).

When an extra one-third atmosphere was applied externally (curve 6) the absorption after one day was slightly greater than in the block with vacuum inside and 1 atmosphere pressure outside. Thus, apparently, about one-third atmosphere was required to neutralize or overcome the increase of pressure in 4 due to compression of the air. Actually the increase of internal pressure must be greater than this, because the calculation is based on the assumption of a complete vacuum in 5, which, as we have seen, was certainly not maintained. The fact that curve 5 ultimately flattens out faster than curve 4 is proof that here, as in treatment 3, there was a slow release of adsorbed gas.

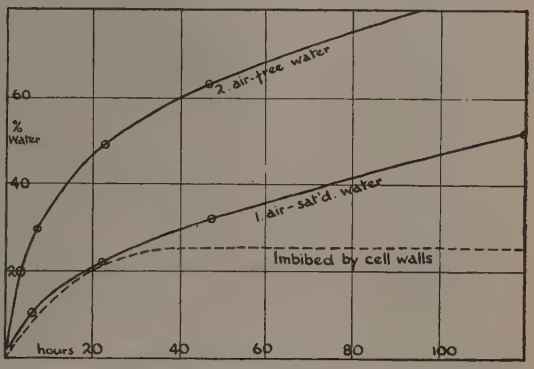

FIG. 4. *Absorption curve of the "free" water into the cavities (calculated by subtracting the imbibed water, estimated from volume-change, from the total).*

The conclusion from these experiments is that the rate of penetration of water into an immersed block of coniferous heartwood under all conditions of pressure and of air content of the water is fixed by the rate at which the air in the block can dissolve and diffuse out.

Application to Logs

Except in high-floating logs subject to evaporation (as already discussed) the condition in logs is similar to that in the present experiments. The water is stagnant, so that the gas is carried away almost wholly by diffusion. Rate of sinkage, therefore, will depend on the gradient of concentration of dissolved gas and still more, as we have seen, on permeability of the cell walls.

As to the difference in concentration inside and outside reference may be made to the Introduction to paper III in this series (2). It is unlikely that variations in saturation of the water outside are of much consequence, but variations in the composition of the gas inside are highly important.

Permeability to diffusion of solutions is not quite the same as permeability to flow of water. The line of least resistance to the latter is longitudinally through the sapwood. The concentration gradient of dissolved air, however, varies inversely as the distance travelled, so that diffusion tends to follow the shortest path, that is, radially to the surface of the log. The diameter of the log, the density of the wood, the amount of ray tissue and the length of the ray cells are thus the chief factors which determine permeability in this connection.

Direct measurements of the relation of permeability to wood structure are in progress.

Acknowledgments

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THE DETERMINATION OF CHOLESTEROL IN BLOOD[1]

By John Allardyce[2]

Abstract

The value of both filter paper and cotton batting as supporting media for blood while drying, prior to extraction of the blood cholesterol with chloroform, was examined; both were found to give unsatisfactory results. With each of these two absorbents the extraction was incomplete. A method is suggested for the determination of cholesterol in blood which involves the use of plaster of Paris as supporting medium, and extraction of the ground blood and plaster of Paris mixture with chloroform. The results obtained with this method are higher and more consistent than those obtained with either filter paper or cotton batting.

In connection with the study of "Haematuria vesicalis" (red water) in cattle, an examination of the literature dealing with the determination of cholesterol in the blood revealed several methods, five of which were considered. With the exception of Windaus' gravimetric method (8), all of these were colorimetric, depending on the Liebermann-Burchard reaction for the development of color. The time, labor, and chemicals required would make the Windaus method prohibitive where any large number of determinations are to be carried out. Bloor's method (1) has found adverse criticism (4, 5, 7), because of the high results it apparently gives. In the Myers and Wardell method (6) there is a possibility of error through the loss of material in the transfers involved. Leiboff has suggested a very simple method (2) which eliminates not only the troublesome plaster of Paris of the Myers and Wardell method, but also the transfers. Leiboff makes no mention of drying the blood before extraction. This may not be necessary when using only 0.25 cc. of blood and his specially prepared filter paper discs; however, with larger amounts of blood, (0.5 cc. or 1 cc.), even when proportionately more filter paper was used, drying has been found to be absolutely essential. Values obtained by this method were consistent but decidedly low. Ling (3) has developed a method which, while essentially the same as that of Leiboff, offers several advantages. Twice as much blood is used, resulting in a better color in the Liebermann-Burchard reaction. A small coil condenser is inserted in each extraction tube giving a more compact apparatus and further the blood is thoroughly dried before extraction. However, folding the filter paper after drying, as called for by this procedure, nearly always resulted in the development of a brown color in the chloroform extract. This was due possibly to the dried blood being crumbled during the folding of the filter paper, and subsequently being washed through by the chloroform. The difficulty was overcome by rolling the filter paper to fit the extraction cups, and fastening them with a pin previous to pipetting the blood on to the roll for drying. As with Leiboff's method, the values obtained were consistent, but decidedly low.

Several modifications of Ling's procedure were tried in an effort to obtain complete extraction. The cups carrying the filter paper were fitted with

[1] *Manuscript received May 28, 1930.*
Contribution from the Chemical Laboratories, University of British Columbia, Vancouver.
[2] *Instructor, University of British Columbia.*

siphons so that the dried blood was completely covered by the chloroform which ran out every two minutes. Complete extraction was only obtained after 12 hr., but even then the values obtained were low. The filter paper was replaced by cotton batting, which previously had been freed from any cholesterol by extraction with choroform. Blood dried on cotton batting, and extracted in the manner just described, gave values about 20% higher than when filter paper was used; there was greater variation, however, in the values obtained for cholesterol although the same samples involved yielded no more cholesterol on further extraction. From the appearance of the cotton batting and extracted blood in the one case, and of the filter paper and extracted blood in the other, it seemed reasonable to suppose that some of the blood had at no time come in contact with the choloroform, due to the hard protective surface formed by the outer layer of blood. In the case of the filter paper, the blood was more evenly, but not so widely, distributed, which may account for a more consistent but lower yield of cholesterol. Whether this was the correct explanation or not, it was evident that neither filter paper nor cotton batting could be used as carrier for the desiccation of the blood.

Although plaster of Paris had always been considered rather troublesome, yet it seemed to be the best medium for the purpose. When blood, dried in plaster of Paris, was ground, transferred to extraction shells, placed in Ling tubes, and extracted with chloroform, not only were the values obtained higher, but they could be duplicated.

Experimental

In the preliminary work, for purposes of comparison, cholesterol was determined in the same sample of blood by each of the three methods, (a) Myers-Wardell, (b) Leiboff, and (c) Ling. The results obtained were as follows, the figures in brackets representing the number of determinations in each case:

Myers-Wardell — 120.3 (6); *Leiboff* — 87 (3); *Ling* — 75 (4).

Two points were apparent in these results: first, that extraction was incomplete, and second, that plaster of Paris allowed a more rapid and possibly more complete extraction.

By combining Myers and Wardell's method of drying the blood with Ling's method of extraction, the cholesterol content of a blood sample from a cow was determined to be 118 mg. per 100 cc. Two weeks later, April 30, 1928, blood from the same cow was extracted in like manner but over a longer period, two hours; seven determinations gave an average value of 170.6. Blood, drawn May 14 from the same animal, was extracted for still longer periods with the following results: 177.7 for a 2.5-hour extraction, 181.7 for a 3-hour extraction, and 183.3 for a 15-hour extraction.

In another set of determinations, cotton batting was substituted for plaster of Paris as an absorbent. The cotton batting, freed from any cholesterol by extraction with chloroform, was dried and cut into small cubes. Each cube, after 1 cc. of blood had been pipetted on it, was again placed in the oven at 45° C. In the first extractions the cotton batting was placed on a bent rod

in a Ling tube and extracted for three hours. The cholesterol content determined in this way on blood drawn July 18 had a value of 140.1 (average of seven determinations). As this value seemed low, possibly due to incomplete extraction, the procedure was altered. The use of extraction cups equipped with siphons did not produce complete extraction even after three hours. The time of extraction was then gradually increased to 12 hr. after which the extraction appeared to be complete, further extraction yielding no cholesterol. Since 12 hr. was an inconvenient period, the extractions were run over night, a period of 15—16 hr. Blood from the same cow was drawn on Aug. 9 and 27 and extracted over night; its determined cholesterol content is given in Table I.

TABLE I

CHOLESTEROL CONTENT OF BLOOD DRAWN FROM A COW

Blood drawn on	Cholesterol in mg. per 100 cc. of blood				
Aug. 9	182.	180.7	183.3	185.6	Ave. 182.9
Aug. 27	173.9	166.6	173.1	170.6	Ave. 167.8
	171.3	151.5			

What was the cause of the two low values on Aug. 27? Was it due to incomplete extraction or to destruction through long heating? Continued extraction failed to yield any further cholesterol, and moreover, when known amounts of cholesterol were extracted for 15 hr., complete recovery was obtained in each case.

Continued use of cotton batting, even when the greatest care was given to every detail, failed to give satisfactory results, for while some results checked well, others varied widely without any apparent cause. Further attempts made with filter paper proved no more successful than those with cotton batting. Moreover, the values obtained when filter paper was used were always lower than with cotton batting, as will be seen for two lots of blood reported in Table II. Seven other lots of blood gave similar results, a less complete extraction being obtained in each case than when filter paper was used. With filter paper, the extraction periods lasted 1, 2, 3, or 15 hr., while the 15-hour period only was used with cotton batting.

TABLE II

COMPARATIVE CHOLESTEROL DETERMINATIONS

Cow	Filter paper				Cotton batting
	1 hr.	2 hr.	3 hr.	15 hr.	15 hr.
No. 14	79.4	83	78.5	81.2	105.2
	79.7				106.6
					102.6
No. 20	80	83.6	78.5	87.8	107.7
	80	84.2	73.4	86.6	105.1
	73.6	69.6	78.5		106
	80.6	77.6	88.1		
	80	87.8	83		
	83.9	87.2	81.9		

A comparison of the results obtained with plaster of Paris, cotton batting and filter paper is given in Table III. The higher and more consistent values obtained after 15-hour extraction, using plaster of Paris, are in marked contrast to those obtained with filter paper and cotton batting.

TABLE III

COMPARISON OF RESULTS OBTAINED WITH THREE SUPPORTING MEDIA

Plaster of Paris		Filter paper	Cotton batting
3 hr.	15 hr.	3 hr.	15 hr.
106.4	156	66.7	93
106	156	66	119.3
105	155	59	103.3
110			125
106			81
118			93

When these samples, with the exception of those in the first column, were subjected to further extraction no more cholesterol was obtained. In addition, it was noted that the cotton batting and filter paper were hard after the extraction, whereas the plaster of Paris if again dried was powdery. It would seem then that in the case of the filter paper and cotton batting, the lower values have resulted from a packing and protective effect of the dried blood preventing complete extraction.

Method

About 20 cc. calcined plaster of Paris was placed in a crucible of 30 cc. capacity. By means of a flat spatula, three crevices were made in the plaster of Paris, and 1 cc. blood was pipetted into these crevices. The crucible was then placed in an oven and kept at 45° C. for three hours. After drying, the plaster of Paris and blood were carefully ground; this was accomplished by first transferring to a glazed paper the unused plaster of Paris. Half of the remaining plaster of Paris and dried blood was removed from the crucible, and the grinding carried out with a glass rod in the crucible. After both portions of the dried blood had been powdered and transferred to an extraction shell, traces of dried blood were washed from the crucible with the unused plaster of Paris. The shell was placed on a small glass support in a Ling tube and the bulb of the tube was three-quarters filled with chloroform. A small coil condenser was inserted and extraction carried out on a sulphuric acid bath maintained at approximately 102° C. for 15—16 hr. At the end of this period, the condenser and shell were removed, the tube cooled to room temperature, and the solution diluted with chloroform to 10 cc. The color was developed in the usual manner by adding 2 cc. acetic anhydride and 0.1 cc. concentrated sulphuric acid, mixing, and setting aside in the dark for 10 min. The color thus developed was compared with that of a solution of naphthol-green-B which previously had been standardized against cholesterol. The dye proved very satisfactory, giving good comparisons.

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BLOOD NORMALS FOR CATTLE; SOME PATHOLOGICAL VALUES[1]

By John Allardyce[2], R. H. Fleming[3], F. L. Fowler[3] and R. H. Clark[4]

Abstract

Samples of cattle blood have been analysed and the concentrations of 10 constituents determined, *i.e.*, cholesterol, sugar, non-protein nitrogen, urea nitrogen, amino-acid nitrogen, creatine, creatinine, calcium, inorganic phosphorus and chlorides. The normal range of each of these constituents was established. The diet fed to the cattle influences the cholesterol content markedly. Blood samples of cattle suffering from red water and some other pathological cases have been examined, and in each case the results show the variation of the contents of the various constituents.

In an investigation of "Haematuria vesicalis" (red water) in cattle, it was considered that analysis of the blood of the affected animals might be of some value in arriving at the cause of this disease, about which little is definitely known. As the normal values for the various constituents to be determined were needed before analysis of pathological cases could be of any significance, the first part of this investigation was directed towards establishing the normal ranges for these constituents in healthy cattle. A search of the available literature showed some scattered determinations of glucose, and cholesterol (7) (11) in the blood of cattle, but no comprehensive study of the normal concentrations appear to have been made. Analyses were made of the following constituents, which from investigations in the analysis of human blood, appeared to be of the greatest value in diagnosis: cholesterol, sugar, non-protein nitrogen, urea nitrogen, amino-acid nitrogen, creatine, creatinine, calcium, inorganic phosphorus and chlorides.

As normal subjects 21 dairy cows and heifers from the Jersey and Ayrshire herds of the Department of Animal Husbandry of the University of British Columbia were used. During the investigation records were kept by Dr. J. G. Jervis, veterinary surgeon, and by the Department of Animal Husbandry showing breed, history, date and hour of collecting blood, period between last feeding and taking of the blood sample, temperature, pulse, milk production, butter fat test, pregnancy, date of last calving, feed, etc.

The blood samples were drawn four to five hours after the morning feeding. Blood was drawn from each animal on several different occasions from one to three months apart. Samples of urine were collected at the same time, for examination*. The blood samples were drawn from the jugular vein using a Jacobi bleeding needle or more frequently a small trocar. An oxalate or fluoride-oxalate mixture was used to prevent the blood from clotting.

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TABLE I

ANALYTICAL RESULTS (MG. PER 100 CC.)

Date	Animal	Cholesterol	Sugar	N. P. N.	Urea-N	Amino acid N	Creatine	Creatinine	Calcium	P. as inorg. phosphate	NaCl from halides
Jan. 21, 1929	1	180	47.35	31.7	14.6	6.2	5.48	1.50	10.45	4.275	445
Jan. 28,		170	49.34	32.9	15.4	6.5	4.83	1.34	10.9	4.3	419.7
Mar. 4,		236.8	48.39	37.1	16.0	7.7	4.14	1.52	9.9	2.95	466.5
June 4,		209.4	48.78	43.4	17.2	6.2	4.57	1.65	9.8	3.92	425
Jan. 21, 1929	2	110.7	53.50	28.6	7.2	5.7	5.59	1.50	12.08	4.4	454
Jan. 28,		113.9	50.0	31.0	13.5	5.0	4.85	1.33	9.65	3.31	457.5
Mar. 4,		207.7	52.45	33.2	14.3	6.6	4.62	1.54	11.6	3.17	471
June 4		181.2	54.16	43.3	16.9	5.5	4.38	1.49	10.4	3.48	439
Jan. 28, 1929	3	76.3	68.18	27.0	19.9	5.8	4.84	1.33	9.0	2.67	488
Jan. 30			54.75								
Mar. 4, 1929		270.	44.12	31.7	14.0	6.9	4.59	1.64	10.15	3.69	486
June 4,		178.	56.48	42.4	20.4	5.6	4.63	1.51	10.5	2.98	450
Jan. 28, 1929	4	177.6	50.34	33.7	14.9	6.2	4.52	1.34	10.4	2.99	431
Mar. 4,		236.8	50.34	35.7	14.4	6.4	4.84	1.50	10.5	3.06	462
June 4,		193.4	50.0	40.2	19.0	5.6	4.52	1.54	10.6	3.62	448
Feb. 4, 1929	5	180.8	55.03	36.3	15.7	5.2	4.99	1.61	9.3	3.75	458
Mar. 11,		226.9	55.97	35.2	17.5	8.1	4.50	1.41	9.75	3.15	490
May 27,		179.6	55.09	36.2	17.4	6.3	5.23	1.46	12.2	3.18	487
Feb. 4, 1929	6	128.1	54.27	33.3	13.9	6.0	4.83	1.59	9.5	4.19	445.5
Mar. 11,		191.5	54.35	31.4	12.6	6.0	4.75	1.37	9.3	3.01	488
May 27,		132.2	53.33	38.5	21.3	6.2	4.72	1.45	12.8	3.74	451
Feb. 4, 1929	7	88.4	51.37	37.4	17.4	6.3	4.70	1.69	9.0	4.47	445
Mar. 11,		108.	52.82	35.3	12.7	6.8	4.54	1.42	9.8	3.25	479
May 27,		141.9	50.89	39.5	16.0	7.4	4.29	1.45	12.6	3.86	477
Feb. 4, 1929	8	81.4	64.93	34.1	14.6	5.3	4.43	1.68	9.1	3.49	483
Mar. 18,		116.3	60.0	31.7	16.2	8.3	3.51	1.60	10.4	4.77	494
May 27,		98.52	51.54	26.1	14.5	6.3	4.50	1.39	11.1	2.84	492.5
Feb. 11, 1929	10	119.5	50.4	31.0	16.2	5.6	5.00	1.65	11.6	3.51	475
Mar. 18,		129	52.45	36.8	19.2	8.6	4.16	1.57	10.55	5.83	496
May 27,		200.1	51.68	41.3	20.4	6.5	4.79	1.49	12.1	3.14	473
Feb. 11, 1929	11	87.38	63.94	33.5	17.1	6.2	4.89	1.66	10.4	3.99	486.5
Mar. 18,		102.0	60.48	34.0	15.7	8.4	4.05	1.53	11.1	5.05	489
May 27,		129.0	54.49	25.7	11.4	7.3	4.53	1.61	12.0	5.4	491
Feb. 11, 1929	12	94.4	62.34	33.9	17.3	6.1	4.66	1.77	9.7	4.45	466.5
Mar. 18,		107.	58.14	30.4	14.3	6.1	3.82	1.61	10.65	4.66	492
May 20,		132.5	49.44	29.6	13.5	5.9	4.64	1.36	11.2	4.82	479.5
Feb. 11, 1929	13	130.8	67.26	38.5	15.7	6.5	4.10	1.63	9.8	4.22	475.5
Mar. 18,		98.0	62.50	31.4	17.1	6.0	4.05	1.59	10.	3.97	496
May 27,		100.1	57.19	30.9	13.9	6.4	4.57	1.61	10.9	3.62	489
Feb. 18, 1929	14	104.6	57.25	32.0	12.1	6.9	4.18	1.50	10.8	3.51	495
Mar. 25,		104.7	54.80	32.8	14.5	7.0	3.97	1.40	10.1	5.10	494
June 4,		277.8	61.20	25.6	12.3	5.7	4.05	1.49	10.2	4.10	443
Feb. 18, 1929	15	77.36	63.03	27.2	11.4	5.7	4.74	1.53	10.4	3.69	497.5
Mar. 25,		94.8	55.97	31.2	14.5	6.0	4.87	1.43	10.45	4.65	493
June 4,		233.5	57.42	40.6	17.0	5.0	4.64	1.46	10.5	3.72	465
Feb. 18, 1929	16	94.08	62.50	35.5	16.3	6.3	5.04	1.46	10.5	5.10	488
Mar. 25,		99.5	65.22	32.7	17.1	6.3	4.66	1.47	10.6	4.28	494.5
May 20,		125.	57.20	35.8	19.8	5.7	4.25	1.53	10.2	5.44	489.5
Feb. 18, 1929	17	83.59	64.1	34.4	15.3	6.8	4.91	1.48	9.85	3.70	490.5
Mar. 25,		82.6	63.56	31.1	14.4	6.5	4.82	1.34	10.8	4.87	486
Feb. 18, 1929	18	125.6	56.39	43.4	19.2	7.2	4.74	1.56	10.1	3.42	484.5
Mar. 25,		161.3	60.06	37.5	19.0	6.2	3.58	1.99	9.6	5.35	492
June 4,		160.5	54.70		19.7	5.5	4.74	1.47	10.6	2.86	447
Feb. 25, 1929	19	159.8	61.98	30.8		6.8	3.90	1.50	10.15	4.06	485.5
Apr. 1,		160.7	60.98	34.1	18.0	7.2	4.09	1.33	11.05	5.40	489.5
May 20,		170.2	59.02	28.5	14.0	5.5	3.87	1.54	11.9	5.08	493
Feb. 25, 1929	20	135.	65.79	27.5	10.9	5.8	4.66	1.50	8.7	3.97	480.5
Apr. 5		136.4	56.50	33.4	17.1	6.5	4.20	1.42	9.85	4.44	487
May 27,		146.	56.18	28.2	15.0	7.0	4.41	1.66	12.3	4.20	481
Feb. 25, 1929	21	153.4	72.12	26.8		5.7	5.03	1.53	8.85	4.71	467
Apr. 1,		157.4	73.53	32.4	14.7	6.0	4.12	1.45	9.85	5.75	494
May 13,		157.1	73.10	31.4	13.0	5.6	3.90	1.61	9.95	6.66	481
Feb. 25, 1929	22	134.3	70.75	29.8		5.9	4.53	1.65	9.85	5.80	480
Apr. 1,		123.02	72.12	31.3	14.5	5.5	3.99	1.42	9.80	5.70	484
May 13,		141.7	62.27	35.2	16.0	5.7	4.11	1.51	10.3	6.70	466

Methods of analysis

Each value given in the tables in this paper is the mean of three or four determinations. The methods used for the analyses were as follows: cholesterol—methods of Myers and Wardell (8) and Ling (6) as modified by Allardyce (1); sugar—Benedict (2); non-protein nitrogen—Folin and Wu (5); amino-acid nitrogen—Folin and Wu (5); creatine and creatinine—Folin and Wu (5); calcium—Clark and Collip (3); phosphate—Fiske and Subbarow (4); chloride—Whitehorn (10).

Results of analyses

The results of the determinations, reported as mg. per 100 cc. of whole blood, are given in Table I.

Discussion of results

From the above results the normal range for these constituents of the blood of cattle would appear to be very nearly the same as found for human beings.

TABLE II

NORMAL CONSTITUENTS OF BLOOD

Constituents	Human beings	Cattle
	Range, in mg. per 100 cc.	Range, in mg. per 100 cc.
Cholesterol	150 — 190	120 — 180
Sugar	50 — 75	50 — 65
N. P. N.	25 — 35	25 — 40
Urea N.	10 — 15	12 — 20
Amino-acid N.	5 — 8	5 — 8
Creatine	3 — 7	4 — 5
Creatinine	1 — 2	1.4 — 1.7
Calcium	9 — 11	9 — 11
Inorg. phosphate	3 — 4	3 — 6
Chlorides as NaCl	450 — 500	475 — 525

On analysis (Table I) the blood constituents, except cholesterol, were found in most cases to remain generally within the normal range (Table II). The variations of cholesterol are explained however by reference to the records on date of calving, feed, etc. For example, during the course of the investigations, animals 3, 10, 14, and 15 calved on Jan. 28, 1929, April 5, 1929, April 13, 1929 and March 13, 1929 respectively. In the case of animal 3, the blood drawn on January 28 was taken two hours before she calved. The cholesterol content of these blood samples showed a marked decrease towards the time of calving, and a considerable increase afterwards. In the cases when the blood was drawn from one to two months after calving the cholesterol increased from 100—200%. In the case of animal 15, a sample was drawn 12 days after calving, at which date the cholesterol content had not reached its highest level. Shope and Gowen (9) found a somewhat similar result.

The cholesterol content of the blood was markedly influenced by changes in feed from corn to sunflower ensilage. A comparison of the data given in Table III with the results in Table I will make this clear.

TABLE III

EFFECT OF DIET ON CHOLESTEROL CONTENT OF BLOOD

Date	Ensilage ration	Animals	Cholesterol
Jan. 28 Mar. 4	30 lb. corn 25 lb. sunflower	1, 2, 3, 4	marked increase
Feb. 4 Mar. 11	30 lb. corn 30 lb. sunflower	5, 6	marked increase
Feb. 4 Mar. 11	15 lb. corn 15 lb. sunflower	7	moderate increase
Feb. 4 Mar. 18	20 lb. corn 20 lb. sunflower	8	moderate increase
Feb. 11 Mar. 18	10 lb. corn 20 lb. sunflower	10, 11, 12	slight increase
Feb. 18 Mar. 25	no change in feed	14, 16, 17	no change
Feb. 25 Apr. 1	no change in feed	19, 20, 21, 22	no change

TABLE IV

ANALYSIS OF BLOOD FROM PATHOLOGICAL ANIMALS

Date	Animal	Cholesterol	Sugar	N.P.N.	Urea N.	Amino Acid N	Creatine	Creatinine	Calcium	P. as inorg. phosphate	NaCl from halides	Remarks
Oct. 1, 1929	12	125.5	48.30	29.6	16.8	5.5	4.61	1.66	11.4	4.24	495	Abortion
Oct. 23, 1929	49	100.9	83.93	31.2	14.7	5.5	5.25	1.76	9.9	5.34	507	Blood poisoning
Oct. 29, 1929	50	103.8	74.65	25.8	9.5	6.1	4.48	1.66	9.2	2.39	505	Abortion
Oct. 29, 1929	51	88.5	51.52	26.5	8.8	6.6	4.02	1.53	9.3	3.02	506	Abortion
Oct. 26, 1929	52	122.3	—	30.0	13.9	4.2	3.99	1.81	8.1	3.42	528	T. B. well advanced
Oct. 26, 1929	53	122.9	48.20	34.4	9.7	5.7	3.62	2.05	9.7	5.06	510	T.B.
Nov. 11, 1929	55	94.9	60.00	28.1	—	6.5	5.90	1.80	10.1	1.88	506	T.B.
Feb. 4, 1929	9	88.5	61.32	36.2	18.7	5.7	4.25	1.65	9.05	3.17	474	Malignant growth in left eye
Feb. 11, 1929		83.08	66.67	44.4	33.4	15.2	4.29	1.57	8.9			
Feb. 25, 1929		124.3	55.97	35.1	17.6	6.4	4.49	1.54	9.2		480	
Mar. 11, 1929		121.1	64.66	34.9	15.5	5.6	4.06	1.52	8.45	4.18	481	
Sept. 19, 1929	47	62.3	57.50	32.4	20.3	4.4	5.85	2.12	10.4	3.11	500	Pyelonephritis left kidney removed Sept. 30, 1929. Oct. 4, other kidney infected. Animal killed and autopsy held Oct. 15, 1929.
Oct. 1, 1929		82.6	70.70	30.7	18.0	4.4	5.27	1.99	9.8	3.14	540	
Oct. 9, 1929		63.3	91.10	178.0	126.3	4.7	22.00	8.33	9.5	3.84	520	
Oct. 12, 1929		69.5	81.83	280.0	203.0	5.7	47.50	14.30	10.0	5.15	510	
Oct. 12, 1929		53.9	113.50	326.0	253.0	5.8	47.30	17.90	9.6	7.87	500	

Pathological Animals

The blood of 25 cattle which had either had red water or had been exposed to it was analysed for the same constituents. The findings were, however, negative; in no case were significant variations of the blood constituents apparent. During the course of the investigation blood samples were analysed from cattle suffering from tuberculosis, abortion, blood poisoning, malignant growths and pyelonephritis. The results of these analyses are given in Table IV; in these cases they were usually within the normal range, except in the case of No. 47 where the conditions of the renal system might readily account for the values obtained.

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HAEMATURIA VESICALIS[1]

By R. H. Fleming[2], F. L. Fowler[2] and R. H. Clark[3]

Abstract

Analysis of the blood of cattle affected with Haematuria vesicalis shows its composition, with few exceptions, to be within the normal range. Two remedies have been found to have a beneficial effect upon cattle suffering from this disease; first, a change of drinking water from surface water to Artesian well water, second, the administration of ground coral rock. The surface water, the Artesian well water and the coral rock from the affected areas have been analysed, and from these analyses certain possible causes for the improvement above noted have been drawn; further field experiments are outlined to test these suggestions.

Introduction

Haematuria vesicalis (red water) is a disease of cattle which is characterized by the presence of blood in the urine. It differs from other forms of red water in cattle in that the blood escapes into the urine through bleeding lesions in the bladder, that no parasites have been shown to be the causative agents, and that it occurs only in very restricted areas (7). Locally it occurs in an area, about 60 miles in width on the Pacific slope of the Cascade mountains, running through British Columbia, Washington and Oregon. Within this area the affected cattle are usually restricted to farms on the higher levels or bench lands, which, however, may not be more than a few feet above neighboring farms free from red water.

Investigations have been carried out along many lines but without material success. The possibility of the disease being caused by parasites, plant poisons, mineral deficiency, oxalic acid or faulty metabolism has been considered by various workers (4, 5, 7, 10).

Blood Analysis

In the present investigation, with the hope that light might be thrown on the problem, analyses have been made of blood from a number of cattle that had had the disease or had been exposed to it. Of the affected animals examined, 14 were suffering from the disease in various stages, four had had the disease and recovered, while seven had been exposed to it, but showed no signs of the complaint.

The results of these analyses were compared with those for normal cattle which previously had been determined in this laboratory (1). The normal range for healthy cattle is given in Table I.

[1] *Manuscript received May 28, 1930.*
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TABLE I

BLOOD ANALYSIS

Constituents	Mg per 100 cc. blood
Cholesterol	120 – 180
Sugar	50 – 65
N. P. nitrogen	25 – 40
Urea nitrogen	12 – 20
Amino acid nitrogen	5 – 8
Creatine	4 – 5
Creatinine	1.4 – 1.7
Calcium	9 – 11
Inorg. phosphate	3 – 6
Sodium chloride (NaCl)	475 – 525

The results of the analyses of the samples from the red water cattle are given in Table II. The methods employed were those used previously (1).

TABLE II

ANALYSIS OF BLOOD FROM RED WATER CATTLE

Date 1929	Animal	Cholesterol	Sugar	N.P.N	Urea-N	Amino acid N	Creatine	Creatinine	Calcium	P, as inorg. phosphate	NaCl from halides	Remarks
June 10	23	150.4	56.4	36.0	16.9	6.8	4.15	1.58	10.8	3.18	490	R.W.—Cleared up
June 10	24	140.8	56.08	39.1	19.0	6.8	4.29	1.5	11.9	3.49	482	R.W.—Cleared up
June 10	25	133.5	56.92	38.8	20.9	6.5	4.83	1.5	9.9	4.54	494	R.W.—
June 10	26	90.5	58.11	29.1	14.7	5.2	4.53	1.52	10.2	5.59	497	R.W.—
June 17	27	174.5	45.32	32.8	15.4	6.65	3.93	1.53	9.6	5.26	522	R.W.—Cleared up
June 17	28	132.8	66.08	34.9	19.1	5.7	4.74	1.49	8.9	4.38	556	R.W.—Bad shape
June 17	29	165.3	57.82	39.4	18.5	6.75	4.07	1.49	9.15	4.77	527	N.R.W.—Lived 1st year on R.W. farm.
June 17	30	154.7	65.77	38.8	19.4	6.6	4.33	1.49	9.1	3.96	514	N.R.W.—Lived 1st year on R.W. farm.
June 17	31	185.3	52.28	33.2	12.8	6.3	4.26	1.46	10.5	4.75	504	R.W.—Cleared up
June 24	32	168.2	47.30	34.9	13.9	8.1	4.53	1.47	10.3	4.24	488	R.W.—Intermittant for 2.5 yr.
June 24	33	139.9	43.37	34.6	14.8	7.25	4.86	1.39	10.6	4.80	504	N.R.W.—Dam and great Dam both died of R.W.
June 24	34	183.5	51.65	32.6	15.7	7.1	4.69	1.41	9.8	4.55	482	N.R.W.—Half sister to No. 33.
June 24	35	147.1	45.25	34.1	16.6	6.4	4.68	1.52	10.3	4.23	493	N.R.W.—Half sister to No. 33 and No. 34.
June 24	36	140.7	49.26	34.2	17 0	6.3	4.77	1.56	10.7	5.25	496	R.W.—Appeared June 17, 1929.
July 2	37	174.0	53.23	25.0	10.4	6.4	4.80	1.44	10.2	5.00	511	R.W.—
July 2	38	184.7	57.40	26.9	14.2	7.0	5.18	1.46	10.2	5.27	516	R.W.—
July 2	39	137.9	59.18	26.7	12.9	5.85	5.44	1.53	9.2	2.89	576	R.W.—
July 2	40	137.7	47.55	24.1	9.9	6.1	4.5	1.55	9.8	2.88	499	R.W.—
July 2	41	104.6	56.83	30.8	14.0	7.0	4.82	1.48	10.2	5.79	489	R.W.—
July 8	42	97.2	61.47	28.7	13.1	4.5	5.12	1.49	9.5	3.45	557	R.W.—
July 8	43	111.3	56.0	31.9	15.9	4.8	5.90	1.44	10.5	4.79	538	R.W.—
July 8	44	101.5	49.75	34.8	13.8	5.05	5.04	1.47	9.5	4.54	524	R.W.—
July 8	45	127.2	57.72	40.4	21.9	4.8	4.64	1.62	9.0	6.33	527	R.W.—
July 8	46	158.1	44.95	36.4	18.1	6.0	4.95	1.47	9.6	5.75	500	R.W.—
Nov. 6	54	33.4	251.00	27.8	17.1	2.0	6.94	1.94	8.2	6.90	529	Bull in last stages, killed 6/11/29.

It may be seen from an examination of Table II that the results, with few exceptions, are within the normal range. Case No. 54 was so far advanced that abnormal values might have been expected. The fact that the blood, as revealed by the analysis, is in apparent normal condition may be taken as an indication that the kidneys are not affected by the disease, at least not until the very advanced stages have set in.

Water Analysis

The possibility of the disease being caused by an excess, or deficiency, of some substance in the diet has been suggested by several workers (5, 10, 14). It has been noted on several farms, where the cattle developed the complaint, that on changing the drinking water from surface water to Artesian well water, the cattle recovered in many cases and the appearance of the disease was checked (8). Cattle, suffering from red water, and not too seriously affected, usually recovered on being moved to farms located in areas free from red water (4, 8). This and the fact that it takes from 5-7 yr. for a healthy heifer to develop the disease, point to faulty nutrition as the probable cause of the complaint. The surface and Artesian well water supply, on a farm where marked improvement had been noted on changing the drinking water from the former to the latter, were analysed following the "Standard Methods of Water Analysis" of the American Public Health Association. The results are given in Table III.

TABLE III

ANALYSIS OF SURFACE AND ARTESIAN WELL WATER

Constituents	Creek water R. Wark farm, Parts per million	Artesian well R. Wark farm, Parts per million
N as NH_3	0.035	0.25
Alb. N	0.41	0.05
Chloride	445	765
Required O	9.7	4.3
PO_4	0	0
I	?	1 in 50,000,000
Boric acid	trace	0
Total Fe	1.38	0.155
SO_4	88.0	195
SiO_2	27.4	21.7
H_2S	none	none
Ca	27.1	56.5
Mg	10.8	19.3
Mn	0.45	0.2
Na	161	254
K	8.5	9.4
HCO_3	93.4	174.5
OH	none	none
Total solids	1105.0	1720
Loss on ignition	100.5	87.5

The Artesian well obtained its water supply from below sea level. The most noteworthy difference revealed by the analysis is the increased amount

of salts in the Artesian water, chiefly sodium chloride, carbonate and sulphate, and calcium bicarbonate. Likewise the Artesian water shows a high iodine content, namely 2,000 parts in 100 billion. McClendon (13) reports that 20 parts of iodine in 100 billion is sufficient to prevent goitre.

Coral Rock

Dr. H. Hendriksen (9) of Burlington, Wash., found that the administration of ground South Sea coral rock had a beneficial effect upon cattle. He administered it, along with sodium bicarbonate, in the proportion of two to one, approximately 100 gm. a day. Following upon the findings of Dr. Hendriksen, the Department of Animal Husbandry of the University of British Columbia are carrying out field trials with the coral-bicarbonate mixture and already report apparent beneficial results in the earlier stages of the disease (4, 11).

The ground coral rock obtained from the Marine Products Corporation of Seattle is reported by them to have the following composition:

CaO.....52.90%	Silica.....4.40%
$CaCO_3$.....94.41%	Iodine..... trace
Organic matter.....0.80%	

Iodine is known to be found in the bodies of corals of certain species in the form of di-iodo tyrosine (3, 15). Analysis made in this laboratory of the coral rock used in this investigation showed very small traces of iodine; consequently any di-iodo tyrosine can be present only in very minute quantity. Di-iodo tyrosine is closely related to thyroxin, is known to be present in the thyroid gland (6), and is of physiological importance.

On the slender evidence that iodine was found in the Artesian water, and existed in minute quantities in the coral rock, it was decided to prepare di-iodo tyrosine for field trials. Tyrosine was prepared by the tryptic digestion of casein after the method of Cole (2), and was converted into di-iodo tyrosine by the method of Wheeler and Jamieson (17).

Since the above process of preparation is lengthy, iodized casein was prepared as this has been shown on hydrolysis to contain di-iodo tyrosine (16). The iodized casein, prepared by the method of Liebrecht (12), was extracted with ether to remove the excess of iodine. Field trials will be made with the di-iodo tyrosine, and also with the iodized casein, as well as with potassium iodide. Further work will be done with the latter chemical since it is open to doubt whether red water appears where potassium iodide is being administered for goitre.

The possibility of the disease being due to iodine deficiency is somewhat supported by the fact that farms on which the cattle are affected are invariably on elevated ground where excessive leaching would take place (4). On the other hand, no abnormality of the thyroid glands has been noted in diseased cattle (8, 10, 14). Another argument against the theory of iodine shortage is that, if due to an iodine shortage, the disease would be expected to be far

more widespread than it is, since deficiency of iodine is fairly common. Further, the disease is only found in cattle, and not in other animals.

There is the possibility that the disease is due to a deficiency of some other mineral substance, although it is difficult to understand why such a deficiency should break down the walls of the bladder and not affect the more important organs of the body. The fact that the urine of cattle suffering with red water is acid (5, 14) instead of having its normal alkaline reaction, offers the suggestion that the action of the coral-bicarbonate mixture may be simply to neutralize this acidity. The same explanation would account for the curative action of the Artesian water, since it contained a higher percentage of sodium and calcium carbonates than the surface waters. Possibly the coral merely aids in maintaining a suitable calcium balance. Field trials with pure calcium carbonate, and also with sodium bicarbonate, in place of the coral rock, will demonstrate this point. Careful analysis of the urine and the acid-base balance of the blood of the red water cattle will be investigated throughout the experiments.

Acknowledgment

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STUDIES ON LIGNIN AND RELATED COMPOUNDS
IV. THE NITRATION OF GLYCOL-LIGNIN[1]

By Harold Hibbert[2] and Leo Marion[3]

Abstract

Glycol-lignin at temperatures below 0° C., dissolves completely in a mixture of four parts of acetic anhydride and one part of fuming nitric acid, giving rise to a 125% yield of a product (nitrated-lignin) which has undergone nitration and acetylation. The nitrogen in the product is present partly in the form of a nitro group, an ester of nitric acid, a nitroso compound and in another form not yet determined. A further treatment of this nitrated-lignin with a nitrating liquor of the same composition, followed by heating at 80° C. causes a short initial oxidation, and yields a substance differing slightly from the first and having a C:H ratio almost 1:1. Repetition of the treatment does not seem to alter the percentage composition appreciably, until after the fourth treatment.

Nitrated-lignin can be methylated, the methoxyl content of the product being more than double that of the original nitrated-lignin. It can also be reduced, the resulting product diazotized and coupled with β-naphthol disulphonic acid.

The soluble products of the nitration reaction contain an acidic component which has not been identified.

Introduction

In a previous communication (8) a method was described for the isolation of lignin in the form of a compound which was designated as glycol-lignin. This method involved the use at 100° C. of glycol, containing a trace (0.05%) of dry hydrogen chloride as a catalyst. In glycol-lignin thus prepared and purified, the lignin present, owing to the mildness of the treatment, has probably suffered little or no structural alteration.

The present communication relates to a study of the nitration of glycol-lignin, the nature of the substances arising from the reaction, and of any relationship existing between the nitrated lignin and the structure of the original glycol-lignin.

It was thought that some definite evidence of the aromatic, aliphatic or hydro-aromatic nature of lignin might thus be obtained. The action of nitric acid on lignin from various sources has been investigated only to a limited extent, and no well-defined aromatic substances have, as yet, been obtained in this way. Recently Heuser, Roesch and Gunkel (7) investigated the action of both dilute and concentrated nitric acid as well as of mixtures of nitric acid and sulphuric acid on Willstätter lignin. A "mixed nitrating acid" did not give rise to any oxalic acid, but dilute and concentrated nitric acid yielded 4.2% and 20% of oxalic acid, respectively. Their results have

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been called into question by Hägglund (6). By the action of nitric acid on the same type of lignin, König (13) obtained an amorphous, nitrogenous compound of the composition $C_{19}H_{32}O_{14}N$.

Franz Fischer and H. Tropsch (3) treated Willstätter lignin with 5 *N.* nitric acid and obtained a nitrated lignin with 4.34% nitrogen, 9.72% methoxyl and 10.54% hydroxyl content; it had a molecular weight in phenol of 810 and they assigned to it the empirical formula $C_{42}H_{37}O_{24}N_3$. Acetylation of their nitrated product gave rise to a derivative which yielded four molecules of acetic acid when hydrolyzed. The ease of nitration of lignin is regarded by Fischer and Tropsch as an indication of its aromatic nature, since the formation of nitric acid esters is not to be expected when such dilute acids are used. Nitrated lignin prepared in the same way was further studied by W. Fuchs (5) who considered it to be an isonitrosoketone-hydroxy-carboxylic acid, and from the ratio of carbon to hydrogen, concluded that it had a cyclic character. He found the molecular weight in acetone to be 1765, and calculated the empirical formula to be $C_{65}H_{67}O_{32}N_3$.

The assumption that true nitro-derivatives are obtained from lignin by treatment with nitric acid receives support from the experiments of Phelps (17) who obtained dyestuffs from the product of the nitric acid treatment of lignin-sulphonic acid.

Since glycol-lignin (8), has not been subjected to conditions as drastic as those under which lignin-sulphonic acid and Willstätter lignin are obtained, a knowledge of its behavior towards nitric acid appeared desirable.*

Nitration of glycol-lignin

When treated at $-12°$ C. with a mixture of fuming nitric acid and acetic anhydride, glycol-lignin slowly dissolved. The addition of the reaction product to a large volume of water precipitated a nitrated lignin. This, after purification, was obtained as a light-yellow powder which exploded when heated in a stream of oxygen. With slight variations in procedure, the yield varied from 115-125%, although the composition of the product remained constant, an observation also made by Fischer and Tropsch in connection with their own nitrated lignin. The molecular weight as determined by the Rast method was found to be 720. The nitrated-lignin thus obtained contained 8.48% methoxyl, 3.18% ester-nitrogen (NO_3), 2.57% combined nitro- (NO_2) and nitroso-nitrogen (NO). As the total nitrogen is 8.65%, there is 2.89% nitrogen present in a form as yet unknown.

In the course of the reaction, the lignin had also undergone acetylation, so that the product was a nitrated-acetylated lignin. On hydrolysis, it yielded 21.1% acetic acid. The nitrated lignin when dissolved in ethyl acetate, did not absorb bromine, and therefore probably contains no unsaturated linkages. It is soluble in weak aqueous sodium hydroxide pointing to the presence of at least one free hydroxyl group. It can be methylated, but during the process

* *Since the completion of this work, Küster and Daur (14) have obtained nitrogen-containing products by the coupling action of diazonium compounds with lignin produced by the agency of hydrochloric acid and alcohol, according to Friedrich's method (4).*

one or more acetyl groups are removed as the methylated product yielded only 7.0% acetic acid on hydrolysis. Also, its methoxyl content was found to be 18.64% as compared with 8.48%, in the unmethylated nitrated-lignin.

When nitrated-lignin was treated again, in the same manner, with acetic anhydride and fuming nitric acid at −12° C., and the resulting solution then warmed to 80° C. for five hours, a product was obtained which had a lower carbon content (39.34% as compared with 41.52%) while the total nitrogen, methoxyl and acetyl content remained approximately the same as in the original product. The molecular weight determined by the Rast method was lower, namely 665. The product of this second nitration, like that of the first, contained nitrogen present as ester, nitro and nitroso radicles, as well as in some other form, which it has not yet been possible to identify. The nitroso-nitrogen was in each case very low. This, coupled with the fact that its value varied rather widely (from 0.39 to 0.82%) with the time taken for the determination, and the presence in the molecule of nitrogen in some unknown form, suggests that the product may not be homogeneous. It may possibly be an equilibrium mixture of two or more compounds. Another possibility which, owing to the variability of the nitroso-nitrogen, is less likely than the former, is that the nitroso radical may be the first step in the formation of nitro-lignin, and that it may not have been fully oxidized to the nitro stage. Nitroso phenol has been found present amongst the products of the nitration of phenol (11).

That some oxidation occurs in the course of the second nitration of glycol-lignin is indicated by the brown fumes of nitrogen peroxide liberated, and by the lower carbon content of the resulting product.

Subjecting the nitrated lignin, already treated at −12° C. and at 80° C., to a further nitration at −12° C. and at 80° C., did not change its composition appreciably, the percentage of C and H being altered only slightly, while the nitrogen remained the same. The product was very stable; after a short initial oxidation characterized by the evolution of brown fumes of nitrogen peroxide, no further visible oxidation took place, even after heating for five hours. If, however, the product was precipitated from solution, dried, and then treated with fresh reagent, the same short initial evolution of nitrogen peroxide was observed, but no further apparent reaction took place. Hydrolysis of the product gave rise to 21.6% acetic acid, which was the same as in the two preceding cases. The methoxyl content was slightly lower.

After the fourth treatment, there seemed to be an alteration in the composition of the product. A sample of nitrated lignin prepared at −12° C., and subjected to four consecutive treatments at 80° C. with the mixture of acetic anhydride and fuming nitric acid, contained 10.49% total nitrogen and 8.06% methoxyl. This residue was extremely resistant towards oxidizing agents and was only very slightly attacked by a 5% chromic acid solution in 5% sulphuric acid, after heating for 15 hr. The yield after the fourth nitration was approximately 20% of the lignin originally taken.

From the analytical results, it seems possible to conclude that the first treatment with the acetic anhydride-fuming nitric acid mixture is primarily a nitration and an acetylation. On the other hand, the first treatment at 80° C. is mainly an oxidation process which brings the C:H ratio of the product to about 1:1 and increases the oxygen content to nearly 50%. The second treatment at 80° C. results in an oxidation of the nitrated-lignin with formation of soluble products, so long as the acid is sufficiently strong; a residue is left which appears to be identical with the original product.

The effects of the successive nitrations on the composition of nitrated glycol-lignin may be compared from the summary of the analytical results given in Table I.

TABLE I

ANALYSIS OF THE PRODUCTS OBTAINED FROM GLYCOL-LIGNIN BY REPEATED NITRATION

Product	Percentage composition										
	C	H	O	Total N	N. as NO_3	N. as NO_2*	N. as NO	N. (Not identified)	OCH_3	$COCH_3$ as acetic acid	Mol. Wt.
I. Glycol-lignin nitrated at −12° C. (A)	41.52	3.86	45.98	8.64	3.20	2.57 NO_2+NO		2.87	8.48	21.1	721
II. Product of nitration of A at 80° C. (B)	39.34	3.50	48.52	8.64	3.24	3.24	0.60	1.56	8.43	20.8	665
III. Product of nitration of B at 80° C. (C)	39.12	3.49	48.49	8.90	3.27	2.88	0.39	2.36	7.39	21.6	
IV. Product of nitration of C twice at 80°C. (D)				10.49					8.06		

* *The figures obtained in the determination of nitro-nitrogen may not be very accurate owing to the possible decomposition of the ester nitrogen. However, it has been established (15) that dilute nitric acid, in a heavily buffered solution, is not appreciably reduced by titanous salts, and also that an excess of nitric acid is reduced to nitric oxide by titanous salts when it is present in relatively high concentrations (16). Since the nitric acid produced by hydrolysis of the ester radical is present in very low concentration it appears safe to assume that a small percentage only of the nitric ester is reduced by the titanous chloride.*

By protecting the free hydroxyl groups in glycol-lignin by methylation, prior to nitration, it was thought that acetylation and nitrate formation might possibly be avoided. Glycol-lignin was therefore methylated and nitrated under the same conditions at −12° C. The yield (64.6%) and lower nitrogen content (7.7% as compared with 8.65%) pointed to a lower degree of nitration, and possibly to a greater degree of oxidation, than was the case with glycol-lignin. The molecular weight of the product, determined by the Rast method, was found to be 898. The product had a higher methoxyl content than nitrated glycol-lignin, but notwithstanding prior methylation still contained

1.06% nitric ester nitrogen and an acetyl content equivalent to 16.1% acetic acid determined by hydrolysis. It differed markedly from the product obtained by methylating nitrated glycol-lignin. This latter contains 18.64% methoxyl whereas the former product has a methoxyl content of 10.23%.

Hydrolysis of nitrated glycol-lignin

Nitrated glycol-lignin, obtained by treating glycol-lignin once at −12° C. with the nitrating mixture, lost 26.0% of its weight on hydrolysis at 100° C. with 5% sulphuric acid, yielding a product containing 1.87% nitrate-nitrogen and 2.75% nitro-nitrogen. It still contained 10.30% methoxyl. It is insoluble in potassium hydroxide, and in all solvents suitable for cryoscopic determinations.

That the elementary composition of the various derivatives obtained from glycol-lignin might be better compared, the analytical results have been summarized in Table II.

TABLE II

SUMMARY OF ANALYTICAL RESULTS

Sample	Percentage composition										
	C	H	O	Total N	N. as NO_3	N. as NO_2	N. as NO	N. (not identified)	OCH_3	$COCH_3$ as acetic acid	Mol. Wt.
Glycol-lignin	61.25	6.14	32.61						18.50		
Hydrolysed glycol-lignin	63.27	5.76	30.97						16.42		
Methylated glycol-lignin	63.69	6.38	29.93						26.26		
Hydrolysed methylated glycol-lignin									22.37		
Glycol-lignin nitrated at −12° C. (A)	41.52	3.86	45.98	8.64	3.20	2.57 (NO_2+NO)		2.87	8.48	21.1	721
Hydrolysed (A)	45.56	3.96		not determined	1.88	2.73	0.47		10.30	not determined	
Methylated glycol-lignin nitrated at −12° C.	41.27	3.88	47.08	7.77	1.06	1.77	0.44	4.50	10.23	16.1	
Methylated (A)	44.05	4.32	43.79	7.84	not determined	2.59	1.21		18.64	7.0	

Reduction of nitrated glycol-lignin and preparation of dyestuffs

Glycol-lignin nitrated at −12° C. is soluble in dilute sodium hydroxide and undergoes reduction by treatment of this solution with sodium amalgam. The reduction product when diazotized, and coupled with β-naphthol disulphonic

acid in alkaline solution, yields a dark red solution, possessing tinctorial properties.

Diazotized α-naphthylamine appears to couple with nitrated glycol-lignin, a dye being produced which dyes wool and silk a pink color. This would point to the presence of a benzene nucleus in lignin, or might indicate a pyrane or furane derivative.

The nature of the soluble oxidation products formed in the preparation of nitrated-lignin has not yet been determined. An appreciable amount of acidic substances was produced and this formed an insoluble silver salt, of which the acid equivalent was found to be 45.5; this is very nearly equal to that of oxalic acid. Although the acid (or mixture of acids) obtained by oxidation of nitrated-lignin formed a calcium salt insoluble in water, yet it formed no amide when ammonia was passed through its methyl alcoholic solution obtained according to the method of Hirst and Purves (9). Oxalic acid under such circumstances rapidly deposits a crystalline amide. Owing to the conflicting nature of the evidence adduced, it is not yet possible definitely to exclude oxalic acid.*

The ease of nitration, the C:H ratio of nitrated-glycol-lignin (approximately 1:1), and the formation of dyestuffs from the reduced nitrated-lignin, all support the assumption of the presence of an aromatic nucleus in lignin, while the coupling of nitrated-lignin with diazotized amines supplies evidence of the presence of a phenolic hydroxyl group.

The quantitative side of the investigation, although it probably gives the correct amount of NO_3, leaves somewhat uncertain the relative proportions of NO_2 and NO. For instance, the presence of oxime-nitrogen has not been settled, and the accuracy of the analytical methods used, evidently, cannot be held for certain until the nature of the combination of the nitrogen in excess over that present as NO_3, NO_2 and NO has been determined. The investigation is therefore being extended in that direction.

Experimental

Preparation of nitrated glycol-lignin (A)

Anhydrous glycol-lignin (2.0 gm.) was slowly introduced at −12° C. into 10 times its weight of a mixture of four parts of acetic anhydride to one part of fuming nitric acid (sp. gr. 1.49)**. The nitrating mixture, previously cooled to −12° C. was kept at that temperature and stirred continuously until the lignin had completely dissolved. The solution was kept at 0° C. for three hours and then allowed to warm to room temperature, filtered through asbestos and poured into two litres of ice-water. The light-yellow nitrated compound precipitating out from solution was filtered, washed with water and dried. Yield 2.5 gm. (125%). It was purified by extraction, first with ether and then with chloroform, the former removing 0.4 gm. and the latter 0.1 gm. Nitrated-lignin is a light-yellow, amorphous powder soluble in

* *Mellitic acid has recently been obtained from lignin by oxidation with nitric acid (10).*

** *All the nitrations were carried out with a similar nitrating mixture, four parts of acetic anhydride and one part of fuming nitric acid, sp. gr. 1.49.*

acetone, ethyl acetate, aqueous pyridine, aqueous piperidine, alkalis, and concentrated sulphuric acid; slightly soluble in alcohol and glacial acetic acid, ether and chloroform, but insoluble in benzene. Analysis:— C, 41.46, 41.59%; H, 3.79, 3.93%; N, 8.64, 8.65%; CH_3O, 8.48%; CH_3CO (as HAc), 21.1%; NO_3, 3.21, 3.18%; (NO_2+NO), 2.58, 2.55%; mol. wt. in camphor, 721.

Methylation of nitrated glycol-lignin (A)

Nitrated glycol-lignin (A) (1.25 gm.), prepared as above, was dissolved in 6.25 cc. acetone, and the solution placed in a flask with 6.25 cc. methyl iodide and 6.25 gm. dry silver oxide. The mixture was kept at 50° C. under a reflux condenser for 10 hr. It was then allowed to cool, filtered, and the methylated nitrated-lignin precipitated from solution by the addition of a large excess of ether. The product was filtered, washed with ether and dried. It was obtained as a light-brown amorphous powder insoluble in alkalis. Yield 0.52 gm. Analysis:— C, 43.83, 44.28%; H, 4.36, 4.28%; N, 7.84%, CH_3O, 18.64, 18.64%; CH_3CO (as HAc), 7.0%; NO_2, 2.62, 2.57%, NO, 1.15, 1.27%, NO_3, not determined.

Nitration of methylated glycol-lignin

Completely methylated glycol-lignin (1.9 gm.) was nitrated and isolated by the same procedure as in the case of glycol-lignin. Analysis:— C, 40.66, 41.88%; H, 3.84, 3.92%; N, 7.68, 8.22, 7.30%; NO_2, 1.84, 1.72%; NO, 0.42, 0.45%; NO_3, 1.06, 1.07%, CH_3O, 10.22, 10.24%, CH_3CO (as HAc), 16.1%.

Nitration of nitrated glycol-lignin (A) at 80° C.

Nitrated-lignin (A) obtained as described above was dissolved in a mixture of four parts of acetic anhydride and one part of fuming nitric acid at −12° C. The flask was connected by a ground-glass joint to a water-cooled reflux condenser and the contents gradually warmed to 50° C. at which temperature oxidation set in suddenly, and brown fumes of nitrogen peroxide were produced for a few minutes. The evolution of gas then ceased, whereupon the temperature was increased to 80° C. and maintained there for five hours, no further visible oxidation taking place. After cooling, the solution was poured into about two litres of ice-cold water, and the precipitated product filtered, washed and dried.

In all further treatments of the nitrated-lignin with the nitrating mixture, the same procedure was used. The material isolated after each treatment was purified by extraction with ether and with chloroform. Only small amounts of the product were removed by these solvents. The product obtained in each case was a light-yellow, amorphous powder, soluble in acetone, ethyl acetate, dilute pyridine (with considerable evolution of heat), and concentrated sulphuric acid, very slightly soluble in ether and chloroform, insoluble in benzene.

Analysis:

Product B—obtained by one treatment of glycol-lignin with the nitrating liquor at −12° C., and one treatment at 80° C. C, 39.07, 39.62%; H, 3.67, 3.33%; N, 8.64%; NO_2, 3.28, 3.20%; NO, 0.38, 0.59, 0.82%; NO_3, 3.20, 3.28%, CH_3O, 8.43, 8.56, 8.29%; CH_3CO (as HAc), 20.8%.

Product C—obtained by one treatment at −12° C., and two at 80° C.; C, 39.64, 38.43, 39.29%; H, 3.48, 3.84, 3.14%; N, 8.90%, NO_2, 2.74, 3.01%; NO, 0.39, 0.39%; NO_3, 3.30, 3.25%; CH_3O, 7.27, 7.51%; CH_3CO (as HAc), 21.6%.

Product D—obtained by one treatment at −12° C. and four at 80° C.: N, 10.03, 10.95%; CH_3O, 8.06%.

Hydrolysis of nitrated glycol-lignin

The nitrated-lignin used in this case was obtained by nitrating glycol-lignin once at −12° C. A quantity weighing 0.95 gm. was hydrolysed at 100° C. for 100 hr. with 50 cc. of 5% sulphuric acid. A residue was left which, after drying over phosphoric anhydride, weighed 0.695 gm. (loss 26.8%). In a duplicate experiment, the loss amounted to 25.2%. The dried hydrolysis product was a brown amorphous powder, soluble with difficulty in concentrated sulphuric acid, insoluble in alkalis. Analysis:— C, 45.88, 45.24%; H. 3.94, 3.97%; NO_3, 1.88, 1.87%; NO_2, 2.71, 2.75%; NO, 0.37, 0.46, 0.57%; CH_3O, 10.32, 10.27%.

Examination of the soluble nitration products

Nitrated glycol-lignin, prepared at −12° C. was subjected to a second treatment with the fuming nitric acid-acetic anhydride mixture at 80° C. for five hours. The resulting solution was poured into a large volume of ice water, and the precipitated nitrated-lignin filtered and washed. The filtrate was then examined for soluble oxidation products.

Methods

The filtrate was evaporated to about 100 cc. under reduced pressure, and the distillate discarded. The residual solution was brought almost to the neutral point with potassium hydroxide. Addition of a 2% solution of silver nitrate caused the precipitation of a white curdy salt, which was filtered, washed with water and dried over phosphorous pentoxide in an evacuated desiccator. An amount of nitrated-lignin weighing 1.0 gm. so treated yielded 0.7605 gm. silver salt.

0.0395 gm. of this salt gave 0.0278 gm. metallic silver, corresponding to an equivalent for the acid of 45.5.

About 0.5 gm. of the silver salt was suspended in water and decomposed by a stream of hydrogen sulphide. After removal of the silver sulphide by filtration, and of the excess hydrogen sulphide by boiling, a solution of calcium chloride was added together with ammonium hydroxide until the solution was alkaline, when a white calcium salt separated. No insoluble copper salt was formed, when cupric sulphate was added instead of calcium chloride.

Examination for oxalic acid

The filtrate from another nitration experiment was evaporated under reduced pressure to a volume of 50 cc. A capillary tube, with one end inserted into a side flask of distilled water, was passed through the rubber stopper of the distilling flask and allowed to dip into the liquid. Water was thus distilled through the reaction product until the distillate was no longer acid to Congo red. The distilled water, in the side flask, was then replaced by methyl alcohol and the distillation continued until 500 cc. of alcohol had passed through the distilling flask. The organic acids from the original filtrate were now left in solution in 15 cc. methyl alcohol, to which was added the required amount of a concentrated methyl alcoholic solution of HCl to bring the total concentration to 3% HCl. The solution was esterified, then cooled to 0° C., and a stream of ammonia bubbled through until saturation was reached. No amide separated from the solution even after standing for 12 hr. in a well stoppered flask.

Preparation of dyestuffs from nitrated-lignin

(a) Nitrated-lignin was dissolved in 1% caustic soda, and reduced with about 200 gm. sodium amalgam, added in successive small portions. The reduction product was soluble in both acid and alkaline solution. The alkaline solution was acidified slightly with hydrochloric acid, cooled to 0° C. and diazotized by the gradual addition of sodium nitrite. The solution of the diazo compound, kept at 0° C. was poured slowly into a well-stirred alkaline solution of β-naphthol disulphonic acid. The solution dyed wool and silk a brownish color when mordanted with tannic acid.

(b) The aqueous solution of the diazonium salt, obtained by diazotizing α-naphthylamine, at 0° C. was slowly poured into an aqueous acetone solution of nitrated-lignin, also kept at 0° C. A purplish-red dye formed which precipitated on standing. It dyed wool and silk a pinkish color, when mordanted with tannic acid.

Analytical methods

The determination of nitroso- and nitro-nitrogen was carried out by means of titanous chloride, methylene blue being used as an indicator in each case. The method used was that of Knecht and Hibbert (2, 12), slightly altered, as follows, to make possible the determination of the two forms of nitrogen on the same sample. The sample of nitrated-lignin was dissolved in concentrated sulphuric acid (1-2 cc.) and washed by means of 20 cc. of water into a flask containing 20 cc. of 40% sulphuric acid. The air in the flask was displaced by a stream of carbon dioxide flowing for 5 min., 2 cc. methylene blue solution (colorless) added, and the solution titrated with standardized titanous chloride, in an atmosphere of carbon dioxide, to a blue end point. This titration gave the nitroso-nitrogen of the compound analysed. Without interrupting the stream of carbon dioxide, standard titanous chloride solution (30 cc.) was then measured into the flask, and the solution warmed in the course of 15 min. to 70-75 C. The temperature was kept at that point for 15 min., during which

the reduction was completed; the excess titanous chloride was then titrated with standard ferric alum solution, the temperature being kept constant at 70-75° C. during the titration.

The efficiency and accuracy of the method, as modified, were established by the analysis of mixtures of *p*-nitrosodimethylaniline and *o*-nitrophenol of known composition. Nitrogen in the nitric ester form may possibly interfere with this determination to a slight extent*, but this is not believed to be sufficient to cause any serious error in the value of the nitro-nitrogen.

The nitric ester nitrogen was determined as nitric oxide in the duPont nitrometer, the samples in each case being dissolved in concentrated sulphuric acid. That nitro-nitrogen is not affected by the nitrometer method has been conclusively shown (1). This is not true of a nitro group attached to carbon through nitrogen, but as there is no nitrogen in glycol-lignin, it does not seem likely that such a group was present in the nitrated derivative.

Acknowledgments

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* *See foot-note, Table I.*

PENETRATION STUDIES

THE PATH OF LIQUID PENETRATION IN JACK PINE[1]

By H. Wyatt Johnston[2] and O. Maass[3]

Abstract

Quantitative measurements have been made, for the first time, of the rate at which liquids penetrate into wood in terms of the velocity normal to the wood-liquid interface. The effects upon this rate, of species, previous history, structure, orientation, thickness, nature of liquid, pressure head, temperature and time have been examined and measured. It has been found that wood behaves largely in a reversible manner provided the treatment does not alter its structure. A technique has been devised for maintaining a single chip in an unvarying environment and following the progress of the cooking process by the variation of the rate of flow of the cooking liquor through the specimen. An attempt is made to examine flow in wood from the standpoint of hydraulics, and a structure is suggested which agrees well with existing anatomical information and gives values for resistance to flow which compare favorably with experiment.

Introduction

Knowledge of the time rate of diffusion of liquids through solids is of interest on account of its bearing on the structure of solids. Much light can be cast upon the size and arrangement of the interstitial spaces by such studies, and this often may lead to important applications in the field of surface chemistry, particularly in the case of colloidal systems such as gels. A most interesting field is that presented by gels which take up fluids with swelling.

Woods possess a very complex structure. They are composed of swelling gels arranged in the form of hollow fibres, formed by the development of living cells which have become highly specialized by adaptation to the life processes of the plant. Woods have a gross structure visible to the naked eye or under a lens of low magnification.

Wood is composed of a condensed carbohydrate called cellulose encrusted by a substance known as lignin which confers upon wood its great rigidity and mechanical strength. Cellulose is found to constitute from 50 to 60% of wood. The lignin may be rendered soluble by certain chemical reagents, leaving the cellulose free from contamination. The rates at which these reactions progress are of some importance in the prediction of the best conditions for disintegrating wood into the cellulose fibres which it contains.

It is of interest to study these rates of reaction at various temperatures and pressures and with different reagents at various concentrations. To do this it is necessary to perform the experiments without destroying the structure of the wood, and this necessitates a knowledge of the rate at which liquids diffuse

[1] *Manuscript received May 28, 1930.*

Contribution from the Physical Chemistry Laboratory, McGill University, Montreal. Constructed from a thesis submitted by H. W. Johnston in partial fulfilment of the requirements for the degree of Doctor of Philosophy at McGill University, Montreal.

The problem and method of attack were initially suggested by Dr. J. Campbell, now associated with Canadian International Paper Company, Limited.

[2] *Chemical Engineer, Forest Products Laboratories, Montreal, and holder, at the time, of a studentship under the National Research Council.*

[3] *Professor of Physical Chemistry, McGill University.*

into wood under the conditions which obtain when these reactions take place. As the reactions which govern the disintegration of wood proceed with appreciable velocity only at temperatures above 100° C., the investigation presents considerable experimental difficulty.

Specimens of wood, whose previous history was known, were subjected to fluid pressure in a manner to be described and the time-rate of diffusion measured in various directions with respect to the structure of the wood, under various pressures and using liquids of different composition and concentration. Provision was made for examining the liquids before and after diffusion and for removing and examining the wood specimens themselves.

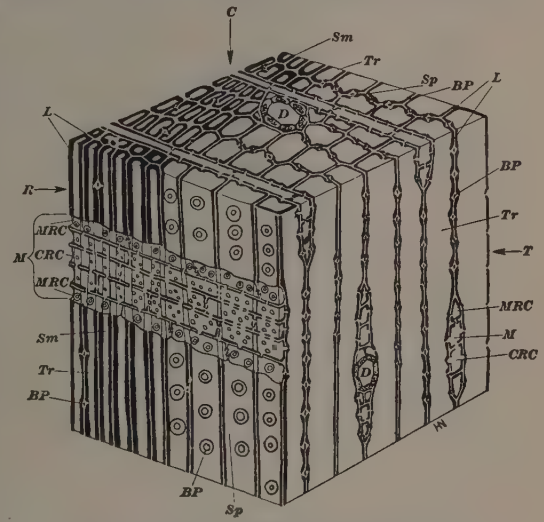


Fig. 1.—Diagram of a Small Block of Spruce Wood, Greatly Enlarged, Showing a Part of One Annual Ring. C, cross section; R, radial section; T, tangential section; D, resin duct; Sm, summerwood; Sp, springwood; Tr. tracheid; BP, bordered pit; L, middle lamella; M, medullary ray; CRC, central ray cell; MRC, marginal ray cell.

In the sulphite cooking process there are two steps occurring more or less consecutively. The first step is the impregnation of the chip with the cooking liquor; the second stage is the solution of the encrusting material by the reagents. If the impregnation is incomplete the disintegration is confined to the outside of the chip, which may even be overcooked while the inner portion remains as a core of imperfectly treated wood. A knowledge of the structure of wood is thus of interest as bearing on the mechanism of the penetration of the wood which must precede a cooking operation.

Wood is composed of hollow axial fibres called tracheids. (For the purpose of this paper, only coniferous woods will be considered.) These tracheids were once living cells, formed by differentiation from the primitive cambial layer just below the bark of the tree.

Communication between tracheids takes place by small openings called bordered pits, whose form is shown in Fig. 11. The membrane or torus of the bordered pits acts as a clack valve. The arrangement in a block of wood is shown in Fig. 1.

There is a layer about one inch in thickness next to the cambium in most conifers which is termed sapwood because in this layer the sap flows freely upward from the roots to the leaves, nourishing the tree. The mechanism of this flow is not known in spite of much study by many workers.

Inside the layers of sapwood is the heartwood, differing little in appearance from the former, but characterized by its resistance to fluid diffusion and its greater strength. Its impermeability is ascribed to changes in the structure of some of the finer organs and to obstructions in the pores of passages by deposits of resins and gums.

Apparatus

The apparatus by means of which observations were made is illustrated in detail in Fig. 2, in which reference numerals are used to designate the several parts which are mentioned in the following description.

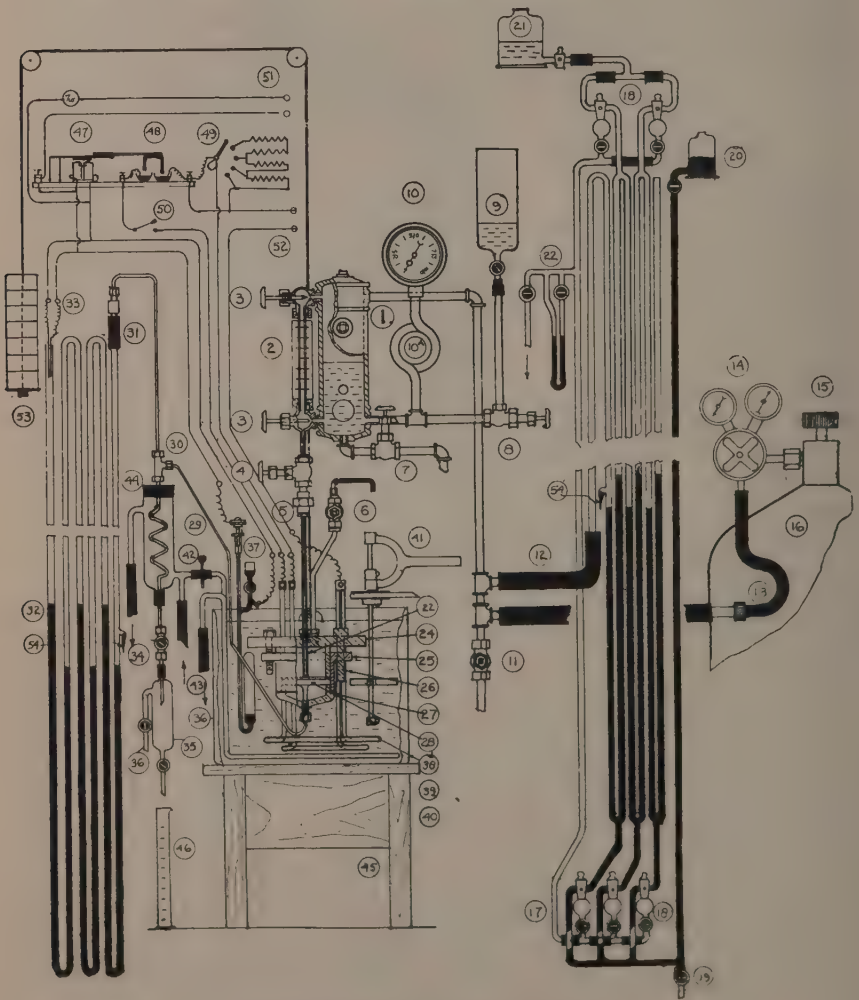


FIG. 2. *Diagrammatic view of the apparatus.*

The manner of making these observations is exceedingly simple. The system is brought to a steady state at the desired temperature and pressure, and the gauge glass is filled by opening valve (3). The lower valve (3) is then closed and valve (4) opened slightly. There is an initial drop of a small amount and the system steadies. The back pressure cock (34) is cracked until the back pressure is stable at a predetermined mark and a reading is taken on the mirror scale, timing the observation with a watch or stop-watch according to the speed of the penetration observed. At some lower value a second reading is taken, the watch stopped and the valve (4) and the cock (34) shut off. The displaced liquid in the receiver is run down and measured. For the slower rates it is easy to manipulate the apparatus single-handed, but for higher speeds the presence of an assistant adds to the accuracy of the work. At very high rates it is almost imperative that assistance be obtained for first-class readings. Otherwise it is best to make (a) a check of input against throughput over a given rate, reading the watch or stop-watch on the move, and (b) to measure either input or throughput, merely keeping count of total input or throughput so that an overall volume check can be made.

This is the method best suited to investigate the laws of flow into wood. There are other methods which have marked advantages and which bear a closer relation to works practice. One of these is to start the penetration going after back-filling and merely equalizing the pressure on the two sides to correspond to the back pressure as shown. The rate of penetration will of course be very slow, and either a reduced size of gauge glass will be needed or a microscope or cathetometer used to read the fall of the free surface. The temperature can be raised at any rate desired and the continuous variation in the rate of flow noted by timing the rate of descent at regular intervals. The sample may be evacuated or not, as desired, before starting the run and at any desired stage it may be blown and the run continued. To wind up, a run in the normal manner may be made. The penetration observed in this method would probably be equal from the two sides, which would give a very fair idea of the actual working conditions in works practice.

In the ordinary type of run it is possible to work up and down the pressure range at each temperature, and thus ascertain what degree of hysteresis exists. Unless it is desired to run with particular rates of temperature increase this method should ordinarily be adopted.

Another valuable experiment is to saturate a specimen in a particular way until the throughput shows that it is well filled and then try the effect of various rates of rise in temperature. This can be done either at constant concentration or at constant fluid content, and is well suited for determining the velocity of the reaction at this temperature. The effect of a change of liquid content is probably equivalent to an extensive blow. A rapid blow followed by a penetration equal to the volume of the specimen and then a second rapid blow would enable the contents of the specimen to be examined by collecting the second blow separately. Dilution from the back pressure gauge would require estimation, but it would permit the determination of liquid which had been in a chip for some time without averaging it with unaltered liquor.

The effect of sugars, etc., and promoters of any kind could readily be studied in this apparatus, while penetration in the vapor phase can also be undertaken by the use of base-free liquors or by saturating a specimen with a base and then treating with a base-free vapor. The procedure would be to run base-free liquor on one side of the chip and a back-pressure on the other side by controlling the cooling coil water.

The apparatus is equally suitable for experiments with sulphate and soda liquors as it is for use with sulphur dioxide and acid sulphites. The operating ranges are slightly different, but the construction of the clamping device and the rest of the apparatus is designed to withstand 175 lb. per sq. in. except in so far as the pressure gauges are concerned. It would not be a difficult matter to erect gauges capable to stand up to 200 lb. per sq. in., especially if the successive legs were made to diminish a little in length at each end, and so facilitate filling under pressure.

Discussion of Results Obtained

Chemical examination of throughput

The general result of the examination of the liquor passed through the specimens is negative. The large volumes employed have a bearing on this; it was the intention that the throughput should not change materially in concentration of reactants.

The analytical agents consisted of 0.2*N*. solutions of iodine, sodium thiosulphate and sodium hydroxide free from carbonate. The solutions were arranged to feed into burettes by gravity from storage winchesters.

Viscosity measurements were made of several of the liquors and an average viscosity relative to water of 1.20 to 1.37 was found. The variation of viscosity with temperature was found to be the same as that found for water, referred to the solution at 25° C.

Results of microscopic examination of samples

The samples examined did not show any very marked difference after water treatment even of a sustained character. There was some evidence of a reduction in lignin, but this was possibly due to the fibres being saturated with water and hence staining more slowly. When samples treated with acid sulphate solutions were examined there was apparently a decided reduction in lignin reaction with phloroglucinol, as was to be anticipated. The results of penetration with mercury are discussed in a special section.

Canada balsam dissolved in xylene seemed to penetrate well into a specimen, and certainly it could be detected farther in than one fibre-length. Specimens penetrated with safranin in solution were found to be thoroughly wetted, but the safranin had not penetrated beyond one fibre-length; it had apparently been filtered out except where it came through in small quantities in an occasional resin duct. The throughput in this instance was much fainter in color than the original solution.

Detailed discussion of experiments run by run

The experiments were conducted with the complete penetrator. The chance discovery of penetration by mercury led to a number of experiments in this field. At this time a series of experiments were made to ascertain if the extractives from wood specimens possessed properties which affected surface tension to any extent. It was found that the effect was extremely small, and quite unlikely to be nearly as much as the normal effect of the components of a sulphite liquor. The experiments were performed by one of the authors with L. M. Pidgeon, with a de Nouy wire ring apparatus. Some of the early runs led to the collection of appreciable amounts of water extractive, but the solutions were so dilute that no appreciable alteration in surface tension or in viscosity could be detected, and the nature of the research did not warrant a chemical investigation of the extractives.

The penetration of wood by mercury and metals

During some early experimentation upon the gaseous contents found in wood, a piece of wood was immersed in mercury by L. M. Pidgeon and one of the authors for the purpose of subjecting it to the action of a Torricellian vacuum. On lowering the mercury the sample was seen to be colored greyish and on removal found to be partially saturated with mercury which oozed out of the wood. The sample was sent to the Botanical Department for investigation and photographing. It was found that the wood, a piece of *Pinus Strobus*, was penetrated at the end for about one fibre-length and at the junction with the sapwood the penetration extended through the specimen. A number of other specimens were prepared at the request of the Botanical Department and the results of the microscopic examination of these by Prof. G. W. Scarth have been published (8).

A sample of wood was also prepared into which a fusible alloy was forced. This to the authors' knowledge is the first communication ever made on this work. The mode of penetration exactly resembles that of mercury.

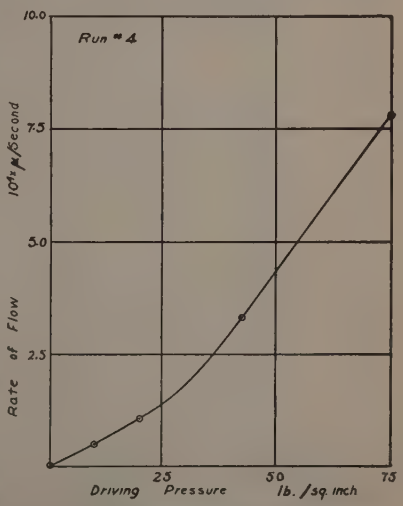


FIG. 3. *The penetration of air through jack pine heartwood.*

It is interesting to notice that in perfectly dry sapwood mercury enters with a flat meniscus, which a little moisture converts to the classical form. In heartwood, penetration by mercury is slow and is practically stopped at the end of one or two fibre-lengths. Continued application of a higher pressure (30 to 75 lb. per sq. in.) will however increase the length of the penetrated portion. Mercury tends to force passages through resin

ducts and to cause wood to split under stronger pressures to an extent not observed with water. No explanation of this presents itself.

The significant features are those pointed out by Scarth. They are the free penetration of sapwood and the very slight penetration of heartwood, the dividing line being so well defined that mercury can be seen on one side of the bordered pit membrane and not on the other. This is an apparent contradiction of I. W. Bailey's statement (1) that a carbon suspension passes through a membrane. Bailey is also able to support his work by photographs of microscopic preparations. There are two considerations which may be advanced at this point. Mercury is not of the nature of wood sap or other liquids found in wood. Although the angle of contact of mercury in dry wood appears to be 90°, there is a tremendous difference between this case and that studied by Bailey. The apertures in a pit membrane are very small so that liquid with a zero angle of contact can get through readily while a very great resistance will be opposed to a liquid making an angle of contact of 180°.

Scarth (8) on the other hand, prefers to consider the tori as flap valves which have become stuck to their seats and so cannot permit flow until they are torn off or dissolved out. The matter will be discussed later in conjunction with flow through fibres of liquids under pressure.

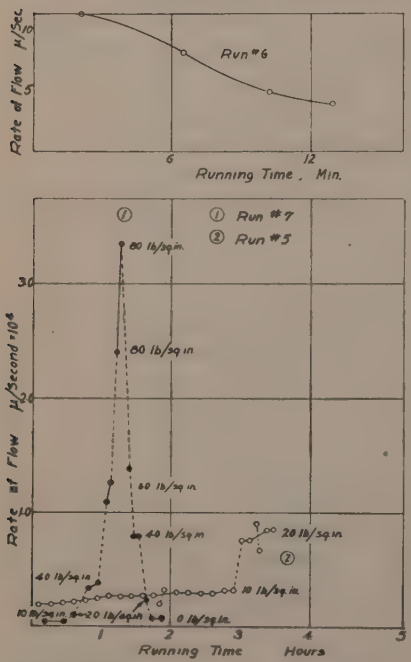


FIG. 4. *Curves illustrating the reversibility of the process of swelling and drying.*

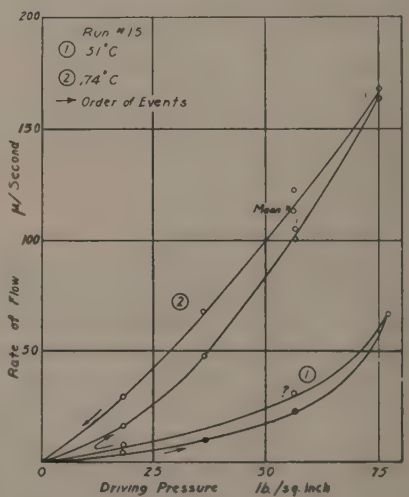


FIG. 5. *Transverse penetration of water in the heartwood of jack pine.*

Condensed tables of experimental values obtained

The rates of flow have been reduced to microns per second.

Run No. 4. Air through heartwood of jack pine 22° C.

lb. per sq. in. gauge	75.0	42.5	20.0	10.0
cm. per sec.	7.77	3.33	1.06	0.50

(= microns $\times 10^4$, per sec.)

This has been plotted in Fig. 3.

Run No. 5. This is shown in Fig. 4. It is evident that the passage of air is drying out the wood and that the rate of flow increases with time.

Run No. 6. Reference to Fig. 4 shows unmistakably how the passage of water causes the rate of flow for water to fall off rapidly toward a minimum value, due to swelling of the fibres.

Run No. 7. Referring once more to Fig. 4, where the results of passing air after Run No. 6 are plotted, it will be seen how the rate of flow for air again increases with increasing exposure, and with increasing pressure, and on diminishing the pressure the values have undergone a marked increase. The inference is that the process of swelling and drying is to a marked degree reversible.

It will not be possible in an abridged paper to present all the results obtained. No attempt will be made to report any but selected runs which are in themselves typical and of interest.

TABLE I

PENETRATION OF WATER IN THE HEARTWOOD AND SAPWOOD OF JACK PINE

Running time			Temperature, in deg. C.	Pressure, in lb. per sq. in.	Rate of flow, in μ per sec.
hr.	min.	sec.			
Run No. 15.					
0	47	40	51.0	18.7	4.12
1	13	40	51.0	36.5	10.00
1	22	20	51.0	56.5	23.3
1	23	20	51.0	74.6	51.85
1	24	23	51.0	77.1	67.0
1	39	21	51.0	18.7	7.87
1	57	47	74.0	18.7	16.35
2	2	57	74.0	36.5	49.0
2	5	3	74.0	56.5	101.4
2	5	48	74.0	75.3	166.0
2	6	42	74.0	56.5	113.7
2	7	24	74.0	36.5	67.9
2	11	52	74.0	18.7	29.7
Run No. 16.					
	6	45	24.0	19.4	270.0
	8	25	24.0	19.4	359.0
	11	15	24.0	19.4	518.0
					Sample subsequently checked

TABLE I—*Continued*

PENETRATION OF WATER IN THE HEARTWOOD AND SAPWOOD OF JACK PINE

Running time			Temperature, in deg. C.	Pressure, in lb. per sq. in.	Rate of flow, in μ per sec.
hr.	min.	sec.			
Run No. 18.					
	20	00	26.0	10.4	0.218
	50	20	26.0	18.96	0.172
1	6	27	26.0	57.43	0.153
Run No. 19.					
	3	53	26.0	78.3	1.39
Run No. 20.					
	2	55	26.0	14.6	215.5
	5	50	26.0	18.96	320.5

NOTE:—*Run No. 15—¼ in. Transverse heartwood* NOTE:—*Run No. 19—½ in. Tangential heartwood*
Run No. 16—¼ in. Tangential sapwood
Run No. 18—¼ in. Radial heartwood *Run No. 20—¼ in. Radial sapwood*
Water was used in each of the five runs.

Runs 15 to 20 bring out very clearly the big difference in rate of flow in the three main directions. In heartwood the rate in a tangential and radial direction is almost nil when compared with transverse penetration. In sapwood there is not much difference in the rate whether the direction be transverse or otherwise.

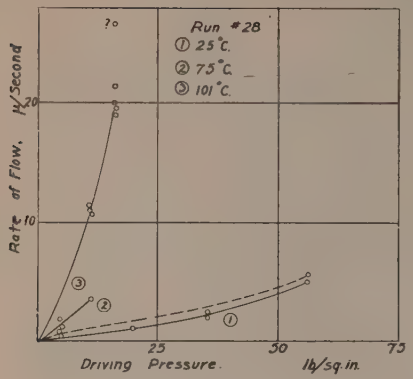
It is convenient to discuss jointly this group of experiments which were made successively upon the same sample of wood to ascertain to what extent different treatments, of not too severe a nature, might alter the rate of flow in wood. A study of the curves (Fig. 5-10) on a pressure base will show that in general the rate increases more rapidly than in a simple ratio to the pressure, especially as the temperature is raised. It would appear that the action of pressure tends to expand the passages through which flow takes place. For the temperature curves it seems best to employ a simple relationship based on experiment, that for solutions actually used in these runs the relative viscosity referred to solution at 25° C. is practically the same as the relative viscosity of water referred to water at 25°. Then the product of the observed value by the relative viscosity will be the rate of flow at 25° C. if the flow is a simple viscous flow, capable of representation in a formula in which the viscosity enters only as a factor. This has been tabulated in Table III. It will be seen that just as in the case of the pressure curves the tendency is for the flow to be greater than viscosity-temperature relations would indicate, especially at the higher pressures and temperatures. The solutions containing sodium sulphite show higher corrected rates, especially when the interpolated figures from higher temperatures are considered in places where errors are con-

TABLE II

PENETRATION OF LIQUID IN THE HEARTWOOD OF JACK PINE

Running time			Temperature in deg. C.	Pressure in lb. per sq. in.	Rate of flow in μ per sec.
hr.	min.	sec.			
Run No. 28.					
	58	28	75.0	4.55	1.78
2	0	50	75.0	4.55	1.22
2	54	32	75.0	10.78	3.59
3	6	17	101.0	10.78	11.01
3	16	13	101.0	15.07	20.08
3	46	13	25.0	4.55	0.90
4	10	33	25.0	56.05	4.94
5	3	33	25.0	56.05	5.53
5	18	33	25.0	56.05	3.85
5	47	27	25.0	34.90	2.40
6	11	57	25.0	19.11	1.09
7	32	37	25.0	4.55	0.455
Run No. 29					
	33	0	26.0	4.40	0.218
2	58	30	26.0	10.90	0.628
3	28	39	26.0	55.03	6.82
5	0	0	26.0	10.45	0.520
6	0	0	50.0	4.32	0.243
8	0	0	49.5	10.43	1.00
8	45	0	50.0	14.91	1.35
9	5	0	50.0	35.11	5.43
10	5	0	75.0	10.40	2.13
10	11	0	75.0	35.11	17.78
Run No. 30					
2	30	0	25.0	10.81	0.410
5	24	0	25.0	14.91	0.460
6	24	0	25.0	15.07	0.452
7	49	0	25.0	34.83	1.16
8	49	0	49.0	10.81	0.662
9	49	0	50.0	15.07	0.937
10	49	0	75.0	10.81	1.29
10	19	0	50.0	34.83	2.75
11	19	0	75.0	15.07	2.17
11	31	30	75.0	34.83	5.73
Run No. 31					
1	0	0	25.5	6.13	0.510
1	30	0	25.5	10.90	0.936
2	0	0	25.5	15.11	1.238
2	29	23	25.5	35.00	3.063
3	0	0	50.0	6.12	0.823
3	30	0	50.0	10.90	1.533
3	50	0	50.0	15.11	2.280
4	0	0	50.0	35.00	6.349
4	35	0	75.0	6.12	1.488
4	45	0	75.0	10.90	2.795
4	55	0	75.0	15.11	4.190
5	5	0	75.0	35.00	12.48
Run No. 32					
	31	0	25.0	6.00	0.656
1	1	15	25.0	15.04	1.848
1	21	15	25.0	35.00	4.50
1	45	0	50.0	6.00	1.084
2	5	0	50.0	15.04	2.920
2	15	0	50.0	35.00	8.118
2	22	0	75.0	6.00	2.195
2	30	0	75.0	15.04	5.505
2	36	0	75.0	35.00	18.35

NOTE:— *Run No. 28—¼ in. Transverse direction; with water.*
Run No. 29—¼ in. Transverse direction; with 5% total SO_2 as Na_2SO_3.
Run No. 30—¼ in. Transverse direction; with water.
Run No. 31—¼ in. Transverse direction; with 4% SO_2 as Na_2SO_3 and 1% free SO_2.
Run No. 32—¼ in. Transverse direction; with water.
The same sample was used throughout these experiments in the sequence given in the Table.



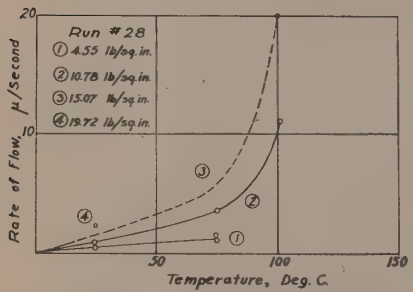
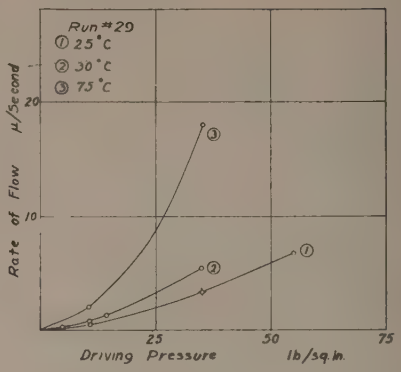


FIG. 6. *Transverse penetration of water in the heartwood of jack pine, at different temperatures and pressures.*



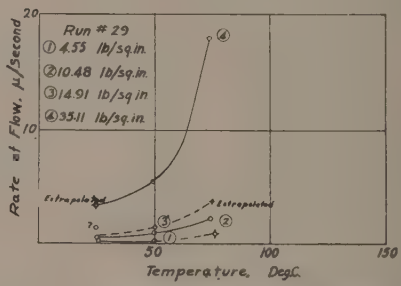


FIG. 7. *Transverse penetration of sodium sulphite solution (5% SO_2) in jack pine heartwood under different conditions of temperature and pressure. The sample was that used in experiments illustrated in Fig. 6.*

ceivable. After the neutral salt the water rate has decreased, on the whole, while after the solution with 1% free SO_2 the water rate is as high as the flow under the influence of the neutral salts.

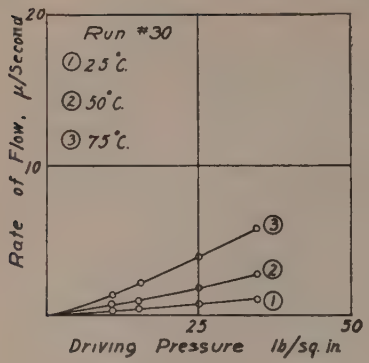
Run No. 35 (Table VII) was made with a typical pulp-mill liquor containing 4.5% total SO_2 and 1% SO_2 as $CaSO_3$. At low pressures and temperatures above 50° C. the results were not valuable, while in any case at temperatures above 50° C., the vapor phase was playing a large part in the flow. Now a reference to Table IV and Table V shows that for 40% free SO_2 at 50° C. the total pressure of the system is about one atmosphere. The applied pressure is probably adequate to prevent vaporization in these cases as the dissolved base will lower the vapor pressure somewhat. The discharge conditions however are just on the border line so the results are of dubious value. At 75° C. the vapor pressure of the system is about 1.9 atmospheres for the 3.5% free SO_2 in the actual solution, so that at least 15 lb. per sq. in. are needed to prevent a vapor phase forming in the inner sleeve, and the discharge will be in the vapor phase. The failure of the results at this temperature to show the anticipated extra rise due to vapor penetration is singular. Possibly deposition of solids in the wood during evaporation may explain it.

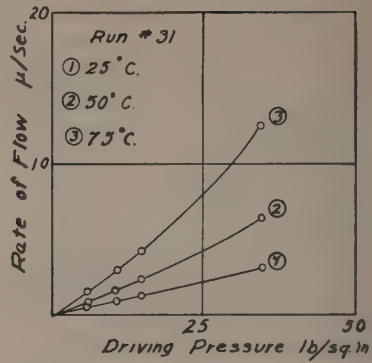
TABLE III

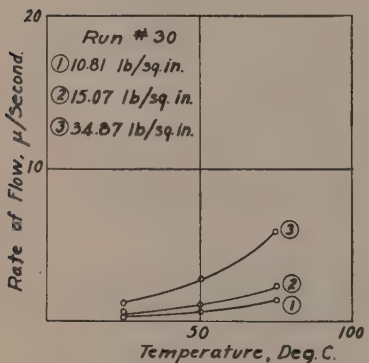
RATES OF FLOW COMPARED DIRECTLY AND AT 25° C.

Temperature in deg. C.	Pressure in lb. per sq. in.	Rate of flow in μ per sec.									
		Run No. 28		Run No. 29		Run No. 30		Run No. 31		Run No. 32	
		Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
25°	5	0.98 0.51	0.515	0.23	0.172 (0.33)	— (0.19)	— (0.24) (0.25)	0.416	0.420 0.517	0.647	0.565 0.780
	10	—	1.45	0.555	0.597 0.868	0.385	0.414 0.507	0.858	0.881 1.095	— (1.15)	— (1.36)
	15	— (0.90)	3.10 —	1.56	0.843 (2.50)	0.452	0.585 0.920	1.23	1.413 1.77	1.848 —	1.825 2.35
	35	2.40	—	— (3.30)	3.40 7.54	1.16	1.72 2.43	3.06 —	4.01 5.31	4.50 —	5.07 7.82
50°	5	—	—	0.275	0.172	— (0.40)	— (0.24)	0.671	0.420	0.904	0.565
	10	—	—	0.955	0.597	0.662	0.414	1.409	0.881	— (2.00)	—
	15	—	—	1.350	0.843	0.937	0.585	2.26	1.413	2.92	1.825
	35	—	—	5.43	3.40	2.75	1.72	6.42	4.01	8.12	5.07
75°	5	1.21	0.515	—	—	—	—	1.215	0.517	1.83	0.780
				(0.80)	(0.33)	(0.60)	(0.25)				
	10	3.40	1.45	2.04	0.868	1.193	0.507	2.57	1.095	— (3.20)	— (1.36)
	15	—	—	—	—	2.165	0.920	4.16	1.77	5.51	2.35
				(5.90)	(2.50)						
	35	—	—	17.78	(7.54)	5.73	2.43	12.48	5.31	18.35	7.82
100°	5	—	—	—	—	—	—	—	° C.	η_t/η_{25}	η_t/η_{35}
	10	9.80	3.10	—	—	—	—	—	25	1.004	1.00
	15	20.0	6.33	—	—	—	—	—	50	0.614	1.63
	35	—	—	—	—	—	—	—	75	0.423	2.35
									100	0.317	3.16

NOTE:— *Figures in brackets were obtained by interpolation.*







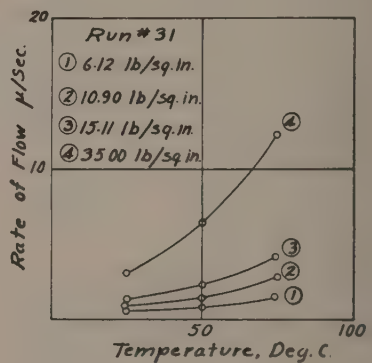


FIG. 8. *Transverse penetration of water in jack pine heartwood under different conditions of temperature and pressure. The sample was that used in experiments illustrated in Fig. 7.*

FIG. 9. *Transverse penetration in jack pine heartwood of a solution containing 4% SO_2 as Na_2SO_3 and 1% free SO_2. The sample was that used in experiments illustrated in Fig. 8.*

TABLE IV

THE TOTAL PRESSURE OF THE SYSTEM SO_2-H_2O

Temperature in deg. C.	Pressure of water vapor	Grams SO_2 per 1000 gm. H_2O			
		10	20	30	40
		Pressure in atmospheres absolute			
25	0.032	0.13	0.23	0.34	0.45
50	0.121	0.34	0.64	0.79	1.04
75	0.39	0.81	1.26	1.65	2.13
100	1.00	1.71	2.44	3.16	3.88
125	2.31	3.39	4.48	5.58	7.06

TABLE V

PARTIAL PRESSURE OF SO_2 IN THE SYSTEM SO_2-H_2O

Temperature in deg. C.	Pressure of water vapor	Grams SO_2 per 1000 gm. H_2O			
		10	20	30	40
		Pressure in atmospheres absolute			
25	0.032	0.095	0.20	0.31	0.42
50	0.121	0.22	0.52	0.67	0.92
75	0.39	0.42	0.87	1.26	1.74
100	1.00	0.72	1.44	2.16	2.88
125	2.31	1.09	2.18	3.17	4.75

(These tables have been condensed from data prepared by W. B. Van Arsdel in I.C.T. Vol. II, p. 302. The effect of the dissolved base has been neglected and the values in the last columns have been extrapolated by the use of Henry's law. The data of Campbell and Maass (3a) were not available at the time these experiments were carried out.

Runs No. 36, 37 and 38 are given in Table VIII. These runs were made to test the effect of thickness. The results are anomalous, as shown by Table VI.

TABLE VI

EFFECT OF THICKNESS

Temperature in deg. C.	Pressure lb. per sq. in.	Run No. 36 (¼ in.)	Run No. 37 (⅜ in.)	Run No. 38 (½ in.)	Run No. 34
25	5	0.65	1.09	1.18	2.50
		2.00	1.97		
	15	2.10	5.51	5.46	
	35	7.27	14.40	13.29	16.60
50	5	1.66	2.054	2.30	
	15	5.96	9.96	9.50	
	35	17.22	25.70	21.37	
75	5	4.23	3.45	3.38	
		6.62			
	15	13.97	15.07	11.46	
	35	35.90	48.20	38.60	

Run No. 36 was made after treatment with sulphite liquor in Run No. 35. The original values of Run No. 34 are not very exact but agree better with the sequence. The difference in thickness is about one fibre-length. The deposition of solids in Run No. 35 has probably spoiled Run No. 36 since the values after treatment (lower ones) are higher and in better agreement.

It is evident on comparing the above values with Table III that the rate of flow in green wood is about four to five times faster than in seasoned wood, indicating that the membranes have been less obstructed.

Runs No. 39 to 46, (Table IX). These runs were undertaken to give a comparison between heartwood and sapwood in green timber for jack pine,

and the same for seasoned spruce. The value for sapwood for instance in Run No. 40 was about 100 times that for the corresponding heartwood in Run No. 39. It is not proposed to compare these runs in detail. It is sufficient to remark that the rate of flow in the heartwood and sapwood of seasoned spruce is of the same order of magnitude as the low values in green jack pine. A few anomalous results were obtained which can only be ascribed to some gross error.

Runs No. 42 and 43 exhibit some irregularity with rising temperature.

In the case of Run No. 42 the result might be due to an error in the temperature at which three readings were taken, but this is not applicable to the results of Run No. 43. There is probably either a gross error in recording or else some effect in the system not fully understood.

Run No. 47. The results of this run have been discussed elsewhere in connection with resin vessels. Runs No. 48 and 49. (Tables X and XI).

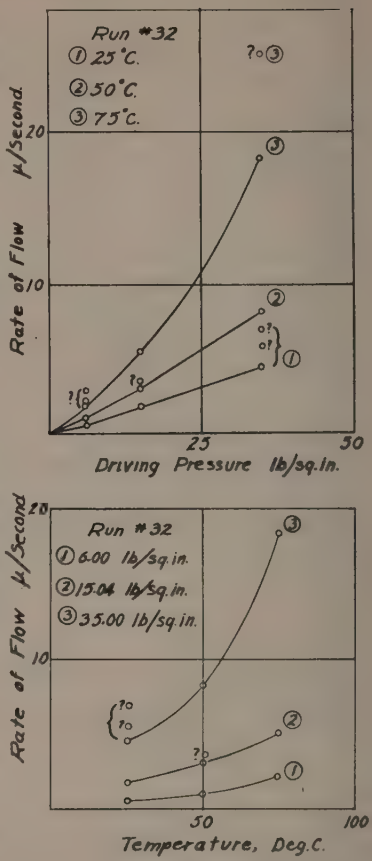


FIG. 10. *Transverse penetration of water in jack pine heartwood under different conditions of temperature and pressure. The sample was that used in experiments illustrated in Fig. 9.*

These runs are of interest from the point of view of technique. They were the first runs carried to the point of disintegration. Run No. 48 was made with pure SO_2 and water in the absence of a base. The pulp was inclined to be dark, but was fully cooked. During the closing period the rate was measured at intervals; it rose rapidly at the close.

The results for a pure NaOH cook (Run No. 49) were similar, except that the cooking effect was only evident toward the close. The pulp was rather dark and slightly undercooked. The reason for closing down was the same, i.e., failure at the edge of the sample. For cooks to disintegration it is obvious that the sample should be held in a bushing and then gripped between gaskets as described above. Leakage appeared quite abruptly but the actual amount was small. A careful examination of the results will show hysteresis effects, with a tendency in any stable state for the pressure to become proportional to the rate of flow.

TABLE VII

EFFECT OF TYPICAL PULP-MILL LIQUOR

Running time			Pressures		Temperature	Rate of flow
			Drive	Back		
hr.	min.	sec.	in lb. per sq. in.		in deg. C.	in μ per sec.
1	0	0	5.00	....	25.0	1.982
	20	0	15.00	....	25.0	14.49
	23	0	35.00	....	25.0	55.93
	27	0	15.00	....	25.0	21.28
2	1	0	5.00	....	25.0	5.68
	11	0	5.00	....	50.0	7.90
	29	0	15.00	....	50.0	29.50
	30	36	35.00	....	50.0	77.10
	37	0	15.00	....	50.0	28.32
	57	51	5.00	....	50.0	7.52
	9	0	15.00	....	75.0	24.87
2	12	0	15.00	....	72.5	22.30
	14	25	35.00	....	75.0	67.35
	16	55	15.00	....	75.0	28.25
2	23	35	66.0	....	100.0	54.50
2	24	39	66.0	....	120.0	313.00
2	27	15	66.0	....	125.0	240.00

NOTE:— *Run No. 35, jack pine, heartwood, transverse direction 0.25 in.*

TABLE VIII

RATE OF FLOW IN THE HEARTWOOD OF JACK PINE

Running time			Pressures		Temperature	Rate of flow
			Drive	Back		
hr.	min.	sec.	lb. per sq. in.		in deg. C.	in μ per sec.
Run No. 36						
1	0	0	5.00	....	26.5	0.645
	26	0	15.00	....	26.5	2.10
	46	0	35.00	....	26.5	7.27
	16	0	5.00	....	50.0	1.66
	27	0	15.00	....	50.0	5.96
	33	0	35.00	....	50.0	17.22
	39	0	5.00	....	75.0	4.23
	45	0	15.00	....	75.0	13.97
	47	0	35.00	....	75.0	35.90
	53	27	66.00	....	75.0	89.30
2	56	26	5.00	....	75.0	6.62
	32	26	5.00	....	26.0	2.25
Run No. 37						
2	5	0	5.00	....	25.0	2.455
	30	0	5.00	....	25.0	1.085
	52	0	15.00	....	25.0	5.51
5	0	30	35.00	....	25.0	14.40
	30	30	5.00	....	25.0	1.346
6	5	30	5.00	....	50.0	2.054
	25	30	15.00	....	50.0	9.96
	30	30	35.00	....	50.0	25.70
	50	30	5.00	....	75.0	3.45
	59	30	15.00	....	75.0	15.07
	2	0	35.00	....	75.0	48.20
	28	0	5.22	....	25.0	1.970
	48	0	5.22	....	25.0	1.845

TABLE VIII—*Continued*

RATE OF FLOW IN THE HEARTWOOD OF JACK PINE

Running time			Pressures		Temperature	Rate of flow
			Drive	Back	in deg. C.	in μ per sec.
hr.	min.	sec.	lb. per sq. in.			
Run No. 38						
1	0	0	5.00	....	25.0	1.180
	31	0	5.00	....	25.0	1.233
	41	0	15.00	....	25.0	5.46
	46	0	35.00	....	25.0	13.29
1	51	0	35.00	....	20.0	12.63
	26	0	5.00	....	50.0	2.296
2	31	0	5.00	....	50.0	2.281
	41	0	15.00	....	50.0	9.50
	48	30	35.00	....	50.0	21.37
	6	30	5.00	....	75.0	3.375
	16	30	15.00	....	75.0	11.46
	21	30	15.00	....	77.5	14.62
	27	0	35.00	....	75.0	38.60

NOTE:— *Run No. 36—¼ in. transverse direction.*
Run No. 37—⅜ in. transverse direction, with water.
Run No. 38—½ in. transverse direction.

TABLE IX

COMPARISON BETWEEN RATES OF FLOW IN GREEN JACK PINE AND SEASONED SPRUCE

Running time			Pressures		Temperature	Rate of flow
			Drive	Back	in deg. C.	in μ per sec.
hr.	min.	sec.	lb. per sq. in.			
Run No. 41						
	35	51	5.00	....	25.0	19.56
	40	57	15.00	....	25.0	109.0
	44	17	10.00	....	25.0	66.70
1	1	8	5.00	....	50.0	32.94
	3	12	10.00	....	50.0	83.50
	4	21	15.00	....	50.0	128.7
	33	24	15.00	....	75.0	56.6
	35	40	15.00	....	100.0	68.0
	42	16	5.00	....	25.0	108.30
	43	35	10.00	....	28.0	221.2
	52	45	15.00	....	28.0	313.0
	54	12	5.00	....	30.0	127.4
2	0	47	15.00	....	32.0	303.6
	18	58	5.00	....	32.0	123.11
	35	9	5.00	....	75.0	162.50
	48	7	5.00	....	75.0	183.6
3	1	30	10.00	....	75.0	318.0
	11	26	5.00	....	100.0	226.2
	16	10	10.00	....	98.0	371.0
	25	26	15.00	....	102.0	446.0
	28	42	15.00	....	125.0	816.0

TABLE IX—*Continued*

COMPARISON BETWEEN RATES OF FLOW IN GREEN JACK PINE AND SEASONED SPRUCE

Running time			Pressures		Temperature	Rate of flow
			Drive	Back		
hr.	min.	sec.	lb. per sq. in.		in deg. C.	in μ per sec.
Run No. 42						
	25	20	5.00	....	25.0	3.69
1	6	22	10.00	....	25.0	9.73
	14	29	15.00	....	25.0	13.51
1	33	50	5.00	....	25.0	5.50
1	42	50	5.00	....	50.0	11.96
1	48	38	10.00	....	50.0	17.00
1	50	30	15.00	....	50.0	43.00
2	2	51	5.00	....	75.0	6.75
2	7	21	10.00	....	75.0	13.15
2	17	21	5.00	....	75.0	7.63
2	20	51	10.00	....	75.0	19.55
2	44	51	5.00	....	25.0	3.085
	14	51	5.00	....	74.0	6.225
	24	51	10.00	....	74.0	13.19
	32	45	15.00	....	74.0	18.18
3	50	35	5.00	....	22.0	14.00
4	2	54	20.00	15.00	100.0	13.78
4	6	32	25.00	15.00	100.0	23.45
	12	32	30.00	15.00	100.0	30.70
4	16	37	37.20	27.20	125.0	13.60
	21	50	42.40	27.20	125.0	27.48
	24	59	47.40	27.20	125.0	39.10
4	26	20	47.40	38.20	140.0	53.10
4	22	17	5.00	....	25.0	1.98
Run No. 43						
	18	21	5.00	....	25.0	3.36
	26	9	10.00	....	25.0	10.37
	17	7	5.00	....	50.0	3.21
	23	10	10.00	....	50.0	10.19
	25	53	15.00	....	50.0	22.62
	44	25	5.00	....	70.0	3.65
	49	23	10.00	....	70.0	9.72
	51	52	15.00	....	70.0	19.86
2	1	52	5.00	....	25.0	4.01
	15	15	5.00	....	75.0	7.41
	19	7	10.00	....	75.0	16.51
	21	46	15.00	....	75.0	32.13
	28	28	20.00	15.00	100.0	19.33
	31	12	25.00	15.00	100.0	38.40
	33	20	30.00	15.00	100.0	57.95
	43	20	32.00	27.00	125.0	14.46
	48	17	37.00	27.00	125.0	24.87
	50	51	42.20	27.00	125.0	43.25
Run No. 44						
	9	15	5.00	....	26.0	367.5
	14	21	10.00	....	26.0	784.5
	20	22	5.00	....	75.0	665.0
	24	24	10.00	....	75.0	946.0
	27	22	15.00	....	75.0	1261.0
	27	37	37.00	31.50	140.0	3740.0

Table IX—*Continued*

COMPARISON BETWEEN RATES OF FLOW IN GREEN JACK PINE AND SEASONED SPRUCE

Running time			Pressures		Temperature	Rate of flow
			Drive	Back	in deg. C.	in μ per sec.
hr.	min.	sec.	lb. per sq. in.			
Run No. 45						
	17	18	5.00	....	75.0	66.3
	25	10	10.00	....	75.0	204.0
	33	22	15.00	....	75.0	220.5
Run No. 46						
	30	30	5.00	....	25.0	0.182
1	1	45	5.00	....	75.0	0.601
1	9	10	10.00	....	75.0	0.332
1	18	23	15.00	....	75.0	0.536
1	30	23	5.00	....	25.0	0.113
1	50	23	10.00	....	75.0	0.617
2	11	23	15.00	....	75.0	0.909
	25	23	37.00	27.00	140.0	5.030
	39	32	37.00	24.50	139.5	5.54

NOTE:— *Run No. 41—⅜ in. Jack pine heartwood and sapwood (complete area); transverse direction, with water.*

Run No. 42—¼ in. Jack pine heartwood; transverse direction; with water.
Run No. 43—¼ in. Spruce heartwood; transverse direction; with water.
Run No. 44—¼ in. Spruce heartwood and sapwood; with water.
Run No. 45—¾ in. Spruce heartwood; transverse direction; with water.
Run No. 46—¾ in. Jack pine heartwood; transverse direction; with water.

TABLE X

EFFECT OF COOK CARRIED TO THE POINT OF DISINTEGRATION*

Running time			Pressure lb. per sq. in.	Temperature in deg. C.	Rate of flow in μ per sec.
hr.	min.	sec.			
	24	50	5.00	25.0	0.497
	40	0	10.00	25.0	1.07
1	7	30	15.00	25.0	0.74
1	20	0	10.00	25.0	0.355
2	13	30	5.00	75.0	0.132
2	48	20	10.00	75.0	0.987
2	58	20	15.00	75.0	1.51
6	25	20	5.00	75.0	0.254
17	49	20	5.00	75.0	0.465
18	33	56	15.00	75.0	2.45
18	44	22	7.00	137.0	31.75
18	51	13	5.00	137.0	29.30
18	54	3	5.00	137.0	35.0

**Run No. 48.*
NOTE:—*½ in. Transevrse jack pine heartwood; 4.5% free and 4.5% total SO_2.*

TABLE XI

EFFECT OF COOK CARRIED TO THE POINT OF DISINTEGRATION*

Running time			Interval in seconds	Fluid input in cc.	Rate of flow in μ per sec.	Pressures		Bath temp. deg. C.	Remarks
hr.	min.	sec.				Drive	Back		
						lb. per sq. in.			
	44	0	2640	1.20	0.2245	5.00	....	25.0	
1	28	0	2640	1.44	0.2695	10.00	....	25.0	
	58	0	1800	1.16	0.3180	15.00	....	25.0	
2	33	0	2100	0.54	0.1268	5.00	....	25.0	
3	3	0	1800	2.83	0.776	10.00	....	75.0	
3	33	0	1800	3.75	1.028	15.00	....	75.0	
3	38	35	335	8.33	12.30	44.00	39.00	145.0	
	42	59	264	7.95	14.85	44.00	39.00	145.0	
3	43	49	50	8.10	79.80	49.00	39.00	145.0	
	44	32	43	5.63	69.60	49.00	39.00	145.0	
3	47	14	167	32.40	95.60	54.00	39.00	145.0	
	50	42	203	48.6	118.0	54.00	39.00	145.0	
3	50	57	15	6.75	222.0	54.00	39.00	145.0	2 p.m.
3	51	57	60	32.0	263.0	44.00	39.00	145.0	4 p.m.
	52	57	60	33.0	272.0	44.00	39.00	145.0	
	53	37	40	66.0	814.0	44.00	39.00	145.0	4.30 p.m. Failure of sample.

**Run No. 49.*

NOTE:—*0.5 in. Transverse jack pine heartwood; 7.5% sodium hydroxide.*

This concludes the run-by-run examination of the results. The general nature of the mechanism of penetration as affected by the factors of temperature and pressure will now be discussed. This requires a special development and it has therefore been placed in a section by itself.

A Discussion of the Mechanism of Flow in Tracheids

Hydraulic theory applied to flow in fibres

Having obtained a series of results for rates of flow, it will be in order to examine whether the regularities observed are susceptible to mathematical treatment. For this purpose a number of principles or propositions have been developed; the detailed discussion of them will be found elsewhere.

The velocity due to a given loss of pressure head

(Throughout this discussion P/w represents pressure head expressed in units of length of water on the C.G.S. system, v and V are velocities in cm. per sec., Z is static head in cm. of water, g is taken as 981 dynes per gm.)

By a well known theorem due to Bernoulli, (4)

$$\frac{\Delta P}{w} = \frac{V^2}{2g}$$

$$= \frac{1}{1033} \times \frac{V^2}{2 \times 981}$$

$$= 0.494 V^2 \times 10^{-6} \text{ atmospheres}$$

$$v = 1.422 \sqrt{\Delta P \times 10^3} \text{ cm. per sec.}$$

These results may conveniently be tabulated.

TABLE XII

VELOCITIES DUE TO LOSS OF PRESSURE HEAD

	0.5	1.0	2.0	3.0	4.0 Atm.
Loss of Head	7.35	14.7	29.4	44.1	58.8 lb. per sq. in.
Velocity	1006.	1422.0	2013.0	2466.0	2844.0 cm. per sec.

LOSS OF PRESSURE HEAD TO YIELD A GIVEN VELOCITY

Velocity	0.2	0.4	0.6	0.8	1.0 cm. per sec.
Loss of Head	0.0197	0.79	0.57	0.315	0.494 Atm. $\times 10^2$

Viscous flow in small tubes

By a well-known relationship due to Poiseuille,

$$P_o - P = \frac{32\,\eta\,lv}{d^2}$$

P = pressure in dynes per sq. cm.

l = length of tube in cm.

d = diameter of tube in cm.

η = coefficient of viscosity in C.G.S. units.

At 0° C. the value of η is 0.01792 dyne sec. per sq. cm. (3)

$$P \text{ atm.} = \frac{0.573v}{1.013 \times 10^6 \times 10^{-8} \times d^2}$$

$$P \text{ atm.} = 56.6\,\frac{\eta_t}{\eta_o} \cdot \frac{v}{d^2}$$

d = diameter of tube in microns = cm. $\times 10^{-4}$

$$V \text{ cm per sec.} = 0.0177\,\frac{\eta_o}{\eta_t} \cdot d^2 \text{ P atm}$$

both results being for a thickness of specimen (i.e., tube length) of 1 cm. The results are most useful when tabulated (Table XIII).

TABLE XIII

LOSS OF HEAD PER CM. PER SEC. PER CM. THICKNESS

η/η_0	Temperature in deg. C.	Diameter of tubes in μ					
		2.0	4.0	6.0	8.0	10.0	100.0
		Loss of head in atmospheres					
1.000	0°	14.15	3.54	1.57	0.885	0.566	0.00566
0.498	25°	7.08	1.77	0.787	0.443	0.283	0.00283
0.307	50°	4.35	1.09	0.48	0.27	0.174	0.00174
0.212	75°	3.00	0.75	0.33	0.19	0.120	0.00120
0.158	100°	2.24	0.56	0.25	0.14	0.090	0.00090
0.124	125°	1.76	0.44	0.20	0.1	0.070	0.00070

Condition for steady flow in fibre

By a well-known relationship due to Reynolds, (4)

$$V_c = \frac{K}{b \times d} \text{ m per sec.}$$

$$(K = \frac{\eta_t \rho_o}{\eta_o \rho_t} = 0.5 \text{ at } 25° \text{ C. } H_2O)$$

($b = 43.8$ for the upper c.v.; b = 278 for the lower (true) critical velocity.)

Tabulating the results obtained for various diameters of tube, as shown above,

TABLE XIV

CRITICAL VELOCITY IN TUBE

	10	100	1000	Microns
Lower V_c'	0.18×10^5	0.18×10^4	0.18×10^3	cm. per sec.
Upper V_c'	1.14×10^5	1.14×10^4	1.14×10^3	cm. per sec.

Assuming a diameter of 30μ (a fair value for tracheids) from Table XIV;

Upper critical velocity = 38×10^3 cm. per sec.

Lower critical velocity = 6×10^3 cm. per sec.

To allow these velocities there is required at least the following pressures;

Upper critical velocity, 775 atm.

Lower critical velocity, 16 atm.,

which are far above the maximum working pressure of seven atmospheres. The conclusion may thus be drawn that flow in tracheids is in general fundamentally stable, and if disturbed will return to the steady state of non-sinuous flow.

Steady flow in a tube fitted with perforated diaphragms

An experiment was made with a fine aperture (ca 50 μ) to ascertain the motion on leaving the orifice of fluid moving below the lower critical velocity. It was found that the motion was stable, the jet expanding to many times its original diameter (ca. 2 mm.) and returning to normal after the production of eddy motion by shock. At higher values than 1 cm. per sec. the jet produced large vortices and broke up. The application of 0.5 atmosphere produced an apparent velocity in the filament after a break of 1 to 2 cm. per sec. When the jet was allowed to impinge on the side of the large tube, it spread out but remained as a sluggish moving sector filling about the lower third of the tube, and not mixing with the upper strata until a 90° bend of four diameters caused complete blending to occur.

The conclusions to be drawn from this experiment are tinged with some uncertainty. It would appear that in a straight tube with diaphragms, flow would take place along a filament bounded by liquid on its periphery and from

10 to 20 times the diameter of the orifice producing it. On the other hand it is not clear that the motion observed was not a train of vortices, and if the path of the filament impinge upon a wall the filament will be spread out over the surface. The filament can bend fairly sharply without becoming sinuous, but it must be remembered that the conditions are not those which are specified in Reynold's formula above and sinuous motion may be produced by disturbances at velocities far below the critical. All that Reynolds formula states is that the steady state will or will not be restored after the disturbing influence has ceased to act, and whether the steady state will or will not break down of its own.

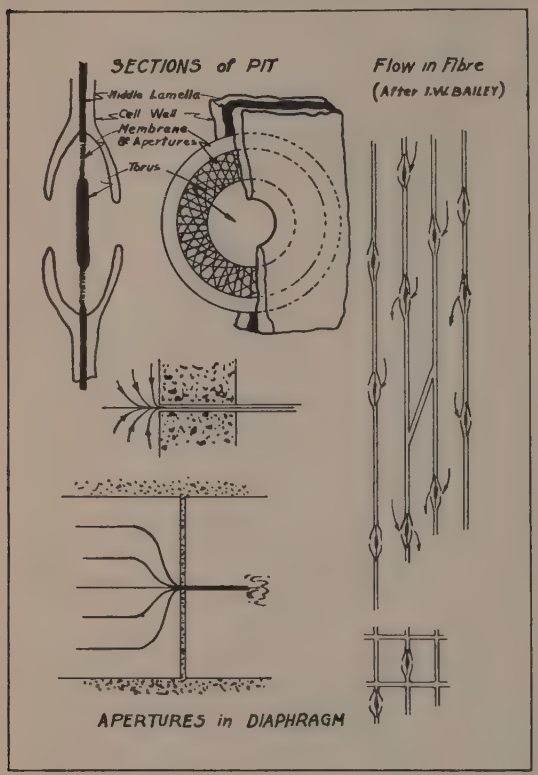


FIG. 11. *Diagrams illustrating the bordered pits in the tracheid walls, and the flow of liquid through the fibres.*

Now consider a tube with a diaphragm and a small hole in it near the centre. (See Fig. 11.)

If A be the area of the tube

a be the area of the aperture (or apertures)

C_c be the coefficient of contraction,

it can be shown that

$$\Delta H = \frac{V^2}{2g}\left\{\left(\frac{A}{aC_c}\right) - 1\right\}^2 *$$

For small apertures and ratios $A : a = 10:1$ take $C_c = 1.0$ and write

$$\Delta P = 0.494 \left(\frac{A^2}{a^2}\right) V^2 \times 10^{-6} \text{ atmospheres.}$$

There is no restriction as to size or location of apertures, but there is the condition that the motion shall be steady. In Table XV is shown the loss of head for various velocities and ratios of cross-section of pipe to cross-section of aperture.

**See Gibson (4), p. 94.*

TABLE XV

LOSS OF HEAD IN ATMOSPHERES FOR NON-SINUOUS FLOW THROUGH TUBES WITH DIAPHRAGMS (VISCOSITY NEGLECTED)

$\frac{A}{a}$	Velocity in cm. per sec.				
	0.2	0.4	0.6	0.8	1.0
	Loss of head in atmospheres				
100	.00020	.00079	.00178	.00316	.0049
200	.00079	.00316	.00711	.01264	.0198
400	.00316	.01264	.02844	.0506	.0790
600	.00711	.02844	.04266	.1138	.1778
800	.01264	.05056	.11376	.2022	.3162
1000	.01976	.07904	.1778	.3161	.4940

If there are m groups of n apertures in parallel the values shown should be multiplied by m/n.

To envisage the significance of this Table XV let a tracheid 30 μ in diameter and 0.3 cm. long (or about three fibres per cm. length) be assumed.

Let the ratio of the area of the openings between two fibres to the area of cross section of the fibre cavity be 1:1000.

From Table XV for $A/a = 1{,}000$, a pressure of 0.49 atmospheres should produce a flow of 1 cm. per sec., or for three fibres in series 1.5 atmospheres would be required.

Actually it has been shown experimentally that two atmospheres will produce a flow of about .0007 to .0015 cm. per sec., according to whether the wood was dry or freshly felled. It is evident that a further explanation must be sought.

Sinuous flow in a pipe fitted with perforated diaphragms

By a well-known theorem in hydraulics the loss in head at a constriction of area a in a pipe of area A in sinuous flow can be shown to be

$$\Delta H = \frac{V^2}{2g}\left(\frac{A}{a.C_c} - 1\right)^2 \quad (C_c \text{ tends to } 0.618)$$

which bears to the expression $\Delta H = \frac{V^2}{2g}\frac{A^2}{a^2}$

the ratio $\left(\frac{1}{0.618} - \frac{a}{A}\right)^2 = 1.618\left\{(1\text{-}1.24\ \frac{a}{A} + (\text{neglected})\right\}$

$= 1.60$ to 1.62 when $\frac{a}{A}$ is greater 0.008.

Table XV may therefore be employed by multiplying the values shown by 1.6 to give the equivalent turbulent motion.

Discussion of the manner in which flow takes place

Having completed the discussion of the ideal cases treated in the five preceding propositions, it will now be in order to attempt to apply the know-

ledge gained to the actual flow in a piece of wood. The structure of wood has been briefly outlined in the introduction, and need not therefore be described here. Reference should be made, however, to Fig. 11 which illustrates the hypothesis advanced by I. W. Bailey (1) that flow takes place through the tracheids which are multiply connected by the bordered pits. Bailey considers that the pit membranes are perforated by minute openings arranged radially around the tori, and in support of this succeeded in preparing photographs of microscopic specimens showing the advance of a suspension of fine carbon particles along the tracheids and the passage of this suspension from one tracheid to the next. An earlier view advanced by Tiemann (9) attributed penetration to flow through the wall of the fibre cavity. G. W. Scarth (8) in a paper read before the Technical Section of the Canadian Pulp and Paper Association, agrees with this so far as flow in sapwood is concerned, but dissents from Bailey's view where flow through heartwood is in question. Scarth considers that in heartwood the tori have been forced over into contact with the pit walls, to which they become cemented by the desiccation and coagulation of the colloidal material in the sap, thus rendering the wood impermeable until the cementing material has been loosened by some solvent or chemical reagent which attacks and renders soluble the resinous material which forms the cement. His basis for this view is that he attempted to force into wood specimens, solutions of water-soluble dyestuffs such as safranin, and found that the average penetration was about one fibre-length. He considers that in the penetration of jack pine the principal cause of the observed penetration is flow through the resin ducts, and that the flow observed in tracheids is due to imbibition in the cellulose material of the wall aided by capillarity. It is proposed to examine this action of the resin vessels in the light of the measurements made by the writer and the preceding discussion of hydrodynamic relations.

Discussion of the effect of resin vessels

These may vary in section from 50 μ to 100 μ; let the latter be assumed. In jack pine 50 have been found to the sq. cm. The resistance would be that of viscous flow.

Referring to Table XIII, the loss of head for a velocity of 1 cm. per sec. and a thickness of 1 cm. is 0.283×10^{-2} atmosphere. The maximum velocity possible under a pressure of one atmosphere will therefore be

$$(0.283\times10^{-2})^{-1}=354 \text{ cm. per sec.}$$

The area of one duct will be $(100\times10^{-4})^2=10^{-4}$ sq. cm. With 50 ducts per sq. cm. the total area is 0.5×10^{-2} sq. cm. and this gives a mean velocity over the section $=354\times0.5\times10^{-2}=1.77$ cm. per sec.

This is more than enough to account for the flow; it is so much more than the observed flow in sapwood that its efficacy is very questionable. It is quite true that by reducing the number supposed to act, the hypothesis might be retained, but of itself it is not a sufficient explanation. There is other evidence against it which can be presented briefly at this point.

1. A sample of wood was carefully clamped and the space between the sample and the back pressure cock (34 in Fig. 2) filled after being exhausted by running in water. Water was then furnished to the other side and it was thought that if the resin vessels played the main part the pressure should rise immediately. Actually a considerable entry (5 cc.) took place before the throughput began to make itself felt. The total throughput was 10 cc. and the total input furnished was 20.5 cc. The gain in weight of the sample was 12.5 gm. water. The straight vertical portion at 3 cc. throughput represents water absorbed by the sample overnight.

2. A sample was penetrated with safranin in the manner described by Scarth (8) but at a temperature of 75° C. The sample was removed after about a half-hour, and dissected. It was found that the safranin had confined itself to the first fibre-length and the resin ducts, but the whole sample was uniformly and thoroughly saturated with water (Run 38).

This water could not have entered through the lower surface, for in the first place it would by hypothesis have been limited to one fibre-length, and in the second place, this run was not made with a back pressure; there was therefore expulsion of the liquid throughout from the bottom of the penetrator, an air lock being present below the sample. It is suggested that the use of material absorbed by fibre so strongly as to dye the material is not a good way to follow the movement of an interface. The use of substances of high molecular weight which are sparingly soluble in water and readily removed by adsorption or absorption for this purpose is to be deprecated.

3. Another sample of heartwood (Run No. 46) was weighed before and after and compared with the sample used in Run No. 47 described above in paragraph 1 of this discussion. The weight of water taken up by a 0.5 in. sample was 12.5 gm. and by a ¾-in. sample 20 gm. very nearly in the ratio of 2 to 3 as required.

4. All the samples which have been dissected (except radial and tangential preparations) have shown themselves to be thoroughly saturated, evidence of which is furnished by the remarkable elasticity and sponginess, and the increased friability which revealed the middle lamella as softened. This effect disappeared as the sample dried out. Where cooking liquors were used the result was even more marked and a uniform cooking effect was detected.

5. It is not denied that flow does occur through the resin ducts, because ocular evidence of that is obtainable in the safranin experiments. What is affirmed is that only a portion of the flow occurs in this way, and that fibre penetration does take place under pressure.

6. It is possible to examine this by the use of the following assumptions:

a. Flow through a relatively large passage such as a resin duct will vary with temperature inversely as the relative viscosity;

b. No wide departure from proportionality to pressure should be observed for such penetration;

c. As resin ducts are of limited length and apparently not all free to conduct liquid, thicker sections should be less affected and there should be a maximum thickness above which the effect of the duct will cease to be noticeable.

7. Now turning to the opposite side of the question, it may be instanced from the illustrations that penetration through the tracheid is fitted to explain the facts as observed.

a. Penetration in dry wood from the pile, while initially rapid, is usually about 25 to 30% of the value observed in green wood. If this were due to open resin ducts, swelling would not produce the marked lowering in rate observed;

b. The smallest apertures are believed to occur in the pit membrane, and it is here that the effect of swelling and drying should be most marked. The result of gas runs before and after water runs is easily explained by the drying out and shrinking of the radial fibres in the pit membranes. It is not easily explained how resin ducts open and shut to the marked extent noticed;

c. Especially in dry or log-pile samples pressure is observed to produce an increase in velocity greater than in simple proportion, whereas viscous flow required direct proportionality and turbulent flow, a relation between the pressure and some power of the velocity higher than the first. This, when taken in conjunction with (*a*) indicates that pressure tends to force apart or lift away surfaces restraining flow, which return to their place on the release of pressure;

d. Hysteresis, often observed in starting a run, is less marked in green wood samples. This is to be expected in tracheid flow and not in vessel flow.

e. Velocity in green wood is more nearly proportional to the applied pressure. This agrees with the hypothesis that wood, which has become seasoned by prolonged drying, has undergone degenerative changes which prevent its reaching the same permeability as green wood, and produces hysteresis besides increasing the time required for such loosening as may occur to take place.

Analytical discussion of the flow of liquids in tracheids

Having thus briefly glanced at the mechanism of the diffusion of liquids through wood and the conflict raised by the hypothesis of Scarth (8), it will be of interest to assume that most of the flow, at least in thicker sections, takes place through the tracheids and, from the existing knowledge of the structure of these fibres, to attempt a mathematical analysis of the resistance to flow in a simplified tracheid for comparison with the observed results.

Tracheids in jack pine and spruce are about 3 mm. long and are hollow, the average section being about 25 μ square inside, or about 30 μ square, counting the share of wall. These figures give an area of gross section (to which the apparent flow relates) of 900 square μ and about three diaphragms in series, with probably from 15 to 30 such sets multiply connected in parallel. It is of interest to develop the analysis in the following proposition on the flow of liquids in small short tubes.

The flow of liquids in small short tubes

It is required to find the flow in tubes less than 0.1 μ in diameter set in diaphragms in larger tubes (Fig. 11) of 900 sq. μ section under a pressure of one atmosphere, when the thickness of the diaphragm is of the order of 1μ.

The formula of Poiseuille, as modified by Knibbs, (5, 6) is:

$$\eta = \frac{\pi g\,(p_1 - p_2)\,R^4 t}{8Ql} - \frac{m\rho Q}{8\pi lt}$$

where Q=discharge in time t, in cc.; ρ=density gm. per cc.; R=radius, $Q = vt\pi.R^2$)

$$\text{or } (p_1 - p_2)\,t = \frac{8Ql}{\pi g R^4}\left(\eta + \frac{m\rho Q}{8\pi lt}\right)$$

$$= \frac{8vl\pi R^2 t}{\pi R^2.gR^2}\left(\eta + \frac{m\rho t R^2 V}{8lt}\right)$$

$$p_1 - p_2 = \frac{8vl\eta}{gR^2}\left(1 + \frac{m\rho R^2 V}{8l\eta}\right) \quad m = 1.13 \text{ (Knibbs)}$$

(For R less than 0.1 cm., v=1 cm. per sec. and l=1 cm.; correction =1½%.)

$$\text{Therefore } P \text{ atm} = \frac{32\,\eta\,vl}{W.gD^2} \text{ (all c.g.s. units)}$$

$$= \frac{32 \times .00894 \times Vl \times 10^{-8}}{1.013 \times 10^6 \times \delta^2 \times 10^{-8}} \quad (w = 76 \times 13.6 = 1033 \text{ gm. } H_2O)$$

with μ as the unit of length.

$P = 28.24\ \frac{vl}{\delta^2} \times 10^{-8}$ atm. for velocity μ per sec. length in μ.

Now taking a large square tube of side 30μ as the outer tube, and letting V be the apparent flow (this includes ½ wall all around).

$$\frac{\pi d^2.v}{4} = 900\ V \text{ and } v = \frac{3600}{\pi\delta^2}\ V$$

$$P = 28.24 \times \frac{3600\ Vl}{\pi\delta^2\delta^2} \times 10^{-8} \text{ atm.}$$

$$= 3.236 \times \frac{V.l}{\delta^4} \times 10^{-4}$$

Assuming three such diaphragms in series, and n such arrangements in parallel forming a multiply connected space,

$$P = 9.72\ \frac{V.l}{n\delta^4} \times 10^{-4} \text{ atm.}$$

Assuming now that V=1.0 μ per sec., l=1.0 μ and δ=0.1 μ

$$P = 9.72\ \frac{1 \times 1 \times 10^{-4}}{n \times 10^{-4}} \quad \text{Let } P = 1 \text{ atm.}$$

$$n = 9.72$$

that is about 10 such orifices are needed for various assumptions as to the value of δ.

TABLE XVI

NUMBER OF APERTURES OF DIAMETER δ PER μ PER SEC.

δ	n	δ	n
0.10	9.72	0.010	9.72×10^4
0.08	23.7	0.008	23.7×10^4
0.06	74.9	0.006	74.9×10^4
0.04	378.8	0.004	$378. \times 10^4$
0.02	6068.	0.002	60.7×10^6
0.01	97200.	0.001	9.72×10^8

From these results it is easy to predict appropriate values for any set of conditions. There is some possibility that the velocity filaments or stream tubes persist unaltered on leaving the aperture, but the experiments of Knibbs (5) indicate that these do not cause a loss of head but only of velocity head and it has been shown above that this is a negligible correction for small velocities. The values shown will vary with temperature as the viscosity varies, and, on the assumption that these are rigid walls, the values will vary directly as the pressure.

The probable mechanism of diffusion in tracheids

On comparing results given in Table XVI, it is interesting to note that they are exactly of the right order or magnitude for comparison with the experimental values. The bordered pits contain the solid centre or torus which, as has been seen, is suspended by a membrane which appears to contain radial markings and is apparently perforated by small apertures. The number and size of these apertures are unfortunately unknown, but it is quite easy to speculate on their possible dimensions. For example, from Table XVI, if the membrane is 1.0 μ in thickness and there are 15 pits connected in parallel on an average, then for an apparent velocity of 1.0 μ per sec. the number of apertures per pit will be inversely as the fourth power of the diameter of the aperture. If this diameter is about 0.06 μ there will be only five apertures per pit, while with a diameter of 0.04 μ there will be 25 apertures per pit and for 0.02 μ diameter there will be about 400 such apertures. If there are 30 pits in multiple the number will be halved.

If the velocity were 10 μ per sec., either there will be more apertures in action or else the diameters will be larger. For instance, with a velocity of 10 μ per sec., and a diameter of 0.1 μ there will be about 100 apertures in parallel, giving over 6.5 apertures per pit. With a diameter of 0.06 μ as before there would be 50 apertures for each of 15 pits in parallel and for a diameter of 0.04 μ there would be 250 apertures per pit. It is easily seen that for a given pressure and number of apertures per pit the diameter of the pit will vary as the fourth root of the velocity, so that the contraction for a tenfold reduction is not very great and would not be easy to observe, even by the most refined microscopic technique.

The effect of a reduction in thickness of the membrane is the opposite. Fewer or smaller pits are then required to perform the function of transmitting the liquid. Here again, however, the diameter for a given number of apertures varies as the fourth root of the thickness, so that a reduction to the thickness of 0.0625 μ will only reduce the diameter requirements to one-half. It may seem that this is strange, considering the normal laws of flow through orifices, but in the types usually examined the thickness is only a small fraction of the diameter and the energy passing through the orifice in unit time is enormously greater than the viscous resistance.

This casts light upon the observed results. The rate of variation is frequently not proportional to the applied pressure, especially in seasoned wood. The explanation is simple. A pressure differential across the diaphragm will tend to displace it away from the pressure and thus produce something resembling homogeneous strain in two dimensions on what may be taken as the surface of a sphere. This will stretch the filaments supporting the tori and enlarge the orifices, permitting increased flow.

Swelling, on the other hand, especially in a dry specimen, can produce an exceedingly rapid diminution in the rate of diffusion, and any substance which affects the swelling of a gel will probably produce an effect upon the diffusion in wood. The action of neutral sulphites is an example of this, the action of hydrochloric acid is another. Knowledge of the isoelectric point would be of value in such a study, and a maximum or minimum rate of penetration with solutions of varying concentration would probably be explicable in this way. All these points are, of course, added evidence in favor of tracheid flow as against vessel flow.

An essential in rapid penetration must be the attainment of conditions which produce minimum swelling of the membranes, or at all events as little as is compatible with the subsequent function of the solution when it has entered the fibres. The ability of solutions to remove incrusted material and soften the membranes is likewise important for this purpose. This is probably why in seasoned wood and even in green wood a rise in temperature causes an increase of velocity out of proportion to the increase in fluidity. The membranes when exposed to moist heat probably decrease in tensile strength, give up water of swelling and yield up more incrusting material which softens or dissolves at the higher temperature. Time is often required after a lowering of pressure before equilibrium is reached and after a lowering of temperature this is always the case. It must be recalled that swelling gels usually return to their initial state if not chemically attacked, but often show marked hysteresis effects.

It is now possible to formulate in a very tentative manner an hypothesis for the diffusion of liquids through wood.

Hypothesis concerning the diffusion of liquids in wood

Diffusion of liquids in coniferous woods takes place largely through the tracheids, passing through minute apertures of the order of magnitude of

20 to 100 $\mu\mu$ in the membranes of the bordered pits. Fifteen to thirty or even more pits probably are multiply connected in parallel and there may be upwards of 100 apertures in a single membrane. The high resistance is due to the exceedingly small size and finite if small length of the tube which forms the aperture. An expression is suggested for the loss of head in terms of the apparent flow over a square centimeter of wood, the number of fibre-lengths in the thickness of the sample, the thickness of the membrane, the diameter of the aperture, the number of apertures in a membrane and the numbers of pits in parallel and the viscosity.

$$P = 9.72 \frac{V.l.L}{Mn\delta^4} \times 10^{-4} \frac{\eta_l}{\eta_{25}}$$

where V = apparent gross velocity in μ per sec.
δ = diam. of aperture in μ
n = apertures per membrane
M = pits in parallel
l = thickness of membrane μ
L = thickness of sample cm.
η = viscosity in C.G.S. units.

The increase of apparent velocity, more rapidly than in simple ratio to pressure, is explained by strain in the membrane.

The decrease of velocity over a period of time is ascribed to swelling.

The increase of velocity, faster than in a simple ratio to the change in viscosity with temperature, is ascribed to weakening of the membrane which makes it deflect and open up more to the loss of water of swelling at higher temperatures and to the softening and removal of incrustations.

Hysteresis is ascribed to the normal properties of swelling gels, and the action of neutral salts upon rates of diffusion, when there has been no permanent alteration in the structure, is set down to the effects of electrolytes in causing swelling or shrinking in gels.

The question of persistence of velocity in the shape of filaments, which would render the problem indeterminate in an alternative solution, is not to be altogether neglected, but Knibbs (5) has shown that it has so far been impossible to detect any other factor, as a corrective to the Poiseuille formula, than the loss of kinetic energy with a suitable coefficient of about 1.13. The possibility of filaments markedly increasing resistance can be neglected until more experimental information is forthcoming.

The question of diffusion through a very thin membrane is really only the limiting case of this hypothesis. The opposite hypothesis, that no flow takes place in heartwood membranes, is negatived by the absence of radial or tangential flow. If penetration occurred by imbibition, penetration would be the same in all directions, as it almost is in sapwood. The difficulty of passage from one tracheid to the next through the intact middle lamella is as great as through the imperforate or sealed-up pit membrane.

Conclusions

The rate of flow is characteristic of the species of wood.

The rate of flow is greater in wood which has been dried than in green wood, but soon falls to a fraction of the latter value.

The flow in sapwood is about 100 times faster than in the corresponding heartwood.

In the axial direction the flow is about 100 times faster than the corresponding flow in the radial or tangential directions, in the case of heartwood, and of the same order of magnitude in the case of sapwood. In jack pine no tangential flow was observed.

The rate of flow diminishes with increasing thickness, but for small thicknesses the effect of resin vessels causes a disproportionate rate of flow to be observed. After the initial stages water, even at 140° C., has but little permanent effect upon the rate of flow.

Neutral sulphites increase the rate at which flow takes place, but no permanent alteration in the rate of flow of water is observed.

Hydrochloric acid reduces the rate of flow very rapidly.

Solutions of sulphur dioxide first reduce the rate of flow and then increase it, leaving at any stage a permanent increase in the rate of flow of water.

Sodium hydroxide produces little effect until high temperatures (ca. 140° C.) are reached when a very rapid effect is produced.

Solutions of acid sulphite cause permanent and continuous increases in the rate of flow of water, but the action is less violent than that of sodium hydroxide.

The rate of flow in greenwood and sapwood tends to increase in proportion to the pressure when the system is in a stable state, but in seasoned wood flow increases more rapidly than pressure, especially at the higher pressures, when even sapwood departs from the linear relationship.

With increasing temperature rate of flow increases more rapidly than fluidity.

The rate of flow of water tends to reach a constant value which does not show a permanent change with time, temperature or pressure. A certain amount of hysteresis is noticed after a marked change in temperature in either direction, especially after a decrease in pressure or temperature when a higher rate of flow is noted than that prevailing at the state before the run. The rate then sinks rapidly to the original value.

Solutions may either produce temporary effects which are restored to normal value upon making a water run, or, as in the case of acid sulphites or other agents such as acids or alkalis at higher temperatures, a permanent increase may occur, increasing with time of exposure until just before disintegration sets in, when a very high value is reached.

There is no evidence of a torus acting as a valve and setting an upper limit or even a maximum value to the increase of flow with pressure.

In an attempt made to examine flow in wood from the standpoint of hydraulics, the structure suggested agrees well with existing anatomical information based on microscopic studies of wood, and gives values for resistance to flow which compare favorably with the experimental values (Fig. 11).

Resistance to flow is caused by minute apertures in the pit membranes, which are in effect capillaries of very short length but of exceedingly fine bore, and these offer sufficient resistance to account for the observed loss of head. It is possible to state how many such apertures of a given apparent diameter would be required on an average per pit membrane. For a given number per pit it is possible to state what the size of the apertures would be on an average for different conditions of temperature and pressure, for different species of wood, for seasoned wood as against green wood, for sapwood as against heartwood and for the change brought about by a treatment of any kind. A mathematical expression is derived which enables these computations to be made from the experimental results.

The hypothesis is expanded to show that pressure would enlarge the openings by distending the membranes, while temperature would act in a twofold capacity by decreasing the tensile strength and by decreasing the sorption of liquids and so the swelling.

Penetration will take place most rapidly at the isoelectric point of the membranes, which could conveniently be determined by measuring rates of flow; that is, maximum flow and maximum penetration correspond with minimum swelling and not with maximum swelling as sometimes stated.

It is suggested that the isoelectric point is probably that of the lignin incrustation, rather than that of cellulose, but the point requires clearing up. It would cast light on the chemical nature of the membrane to study the point and compare the value obtained with direct results on lignin and on cellulose.

It is suggested that the cooking action is preceded by diffusion through the cellulose wall of the fibre, and, by maintaining a constant concentration of reactants in the cell and a sufficient flow to remove products as fast as they diffuse out, it would be possible to investigate the reaction velocity at the interface cell-wall—middle lamella. The problem presents a similarity to a Donnan equilibrium membrane, with one vessel secured against diffusion other than ionic by filling with agar-agar.

The following new contributions have been made to the knowledge of the chemistry of wood:—

1. An apparatus has been built to study penetration and reaction velocities in the interior of blocks of wood.

2. A technique has been devised for measuring the effect of the environment on the rate of diffusion of liquids through wood.

3. A technique has been devised for the study of reaction velocities in the disintegration of blocks of wood under the influence of reagents at constant concentration.

4. A technique has been devised for following the progress of disintegration of wood in blocks by the rate of diffusion of liquids through the specimen during the reaction.

5. A structure has been suggested and an hypothesis advanced which is sufficient to explain the resistance to flow of liquids offered by wood, and to account reasonably for the manner in which this flow varies with temperature, pressure, time, composition and concentration of liquids, species of wood and historical and anatomical condition of the specimen, and to assign numerical values, agreeing well with experiment, to the dimensions of the apertures in pit membranes given the apparent velocity of diffusion through the specimen under known values for the factors named above.

6. A new and simple variation of the series manometer has been devised and built of glass; it can be designed to read absolute pressures up to 15 atmospheres.

7. A device has been made for clamping specimens which it is desired to subject to diffusion.

8. Information on the apparent velocity of diffusion of liquids through wood has been observed and recorded. It is thought that this information will be not only of scientific interest but may be of interest to the manufacturers of wood pulp.

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ENERGY LOSSES AND SCATTERING OF ELECTRONS IN MERCURY VAPOR[1]

By D. C. Rose[2]

Abstract

Experiments have been carried out in which the energy losses of electrons striking mercury atoms have been studied. The apparatus was so designed that scattering relations could be studied for elastic and inelastic collisions.

Energy losses representing the 4.7 or 4.9-volt energy level, the 6.7-volt energy level, the 10.4-volt level, or ionization, and some indication of intermediate levels have been found. Energy losses greater than the ionization potential have also been found at 11.5 and about 13.5 volts. The latter is without doubt the result of two successive collisions each resulting in the loss of 6.7 volts energy. The former due to its intensity cannot be explained as the result of two successive collisions, and its significance is discussed. Similar experiments by Whitney and Foard are discussed.

The scattering curves are not as accurate as could be wished, due to an insufficient number of observations having been taken. They show, however, that the scattering is not of the type of the inverse square law of force. This is in agreement with observations by Arnot.

Recently many important experiments have been performed in which the energy losses were measured when electrons collide with atoms of a gas or vapor. The general method was to define a beam of electrons from a filament by slits, the filament and slits forming what is commonly called an electron gun. The electrons then passed into a field-free space filled with the gas or vapor to be studied. Here the collisions occurred and a second set of slits defined a "scattered" beam of electrons. From this pair of slits the electrons passed into an "analysing chamber" where their energy distribution could be measured. There are three general types of analysing chambers which will be discussed in later paragraphs.

In general the following type of results have been obtained by various experimenters. When the velocity distribution of electrons scattered through a fixed angle is measured, peaks occur in the velocity-numbers of electrons curve which correspond to: (a) Elastic collisions, that is, electrons which have been deflected without loss of energy. (Of course if the slits in the electron gun and those defining the scattered beam are in line—scattering angle θ -then the elastic peak includes those electrons which have passed through without collision.) (b) Inelastic collisions, or a series of peaks is found in the curves which correspond to those electrons which have given up a definite amount of their energy to the atom. For instance in helium, Dymond and Watson (3) found peaks in their curves corresponding to energy losses representing excitation and ionization potentials.

Hughes and McMillan (8) in a preliminary note recently published, report similar experiments made on helium, using the electric rather than the magnetic

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method of analysis. They found peaks corresponding to the 21.12, 22.97, 23.62-volt energy levels but none at the 19.77 and 20.55-volt levels. The initial energy of their bombarding electrons was 100 volts. Harnwell (6) has also worked on energy losses in helium and other gases, using the electric method of analysis, but the resolving power was not very high. In hydrogen, Jones and Whiddington (10) found peaks corresponding to energy losses of 12.6 and about 9 volts. From band spectra data the first of these peaks can be said to correspond to one of the molecular energy levels as found from spectroscopic studies of hydrogen. The second peak (9 volts) which occurs at low voltages around 15 to 25 volts, is interpreted as indicating a collision resulting in dissociation with some energy omission in the form of continuous spectrum. Roberts and Whiddington (11) using the same apparatus also investigated oxygen. As will be seen the interpretation of such experiments on molecular gases is always much more difficult than in the case of atoms, but in both cases this type of experiment promises to yield important information on atomic or molecular structure of a nature that cannot be obtained spectroscopically.

In the experiments to be described here the energy losses in mercury vapor were studied and it will be seen that results were found which for the most part can be correlated with the known energy levels of the mercury atom. While these experiments were being reported in the form of a preliminary notice (12) papers appeared by Foard (5) and Whitney (13) describing experiments on energy losses of electrons in mercury which gave essentially similar results as those found by the author. However, though the present experiments are still incomplete the method employed is sufficiently different to warrant a review of the results in their present state. Considerable space will be devoted to the description of the apparatus and the method of procedure as several important technical features were involved.

Apparatus and Method of Experiment

The electron gun

When homogeneous beams of electrons of slow velocities are desired, the electron gun is always one of the important features of the apparatus. The type of gun used in the present experiment was similar to that designed by Farnsworth (4). The electrons from a hot tungsten filament were first accelerated by slit A, Fig. 1, and then retarded in the space between slits A and B. Then slits B and C defined the beam of electrons used. The filament was shaped so that the current in it produced a minimum magnetic field. The dimensions of this electron gun were considerably smaller than those used by Farnsworth. The diameter of the cylinders separating slits A and B and B and C was 5 mm. so that slits A and B were 5 mm. apart and slits B and C about 15 mm. apart. Slit A was about 0.75 mm. by 3 mm. and slits B and C about 0.3 mm. by 3 mm. All the slits were cut in copper as it seemed the most satisfactory non-magnetic material. With this gun a homogeneous beam of electrons could be obtained easily, having energies as low

as six volts and an intensity of the order of 0.1 microamp. through slit C. The filament current was about four amperes. The magnetic field of the filament prevented lower energy beams from being obtained in a satisfactory manner. The electron gun and analyser were contained in two bulbs each about 3.5 in. in diameter. In a new apparatus which is to be constructed it is hoped that electron beams will be obtained having energies of less than one volt.

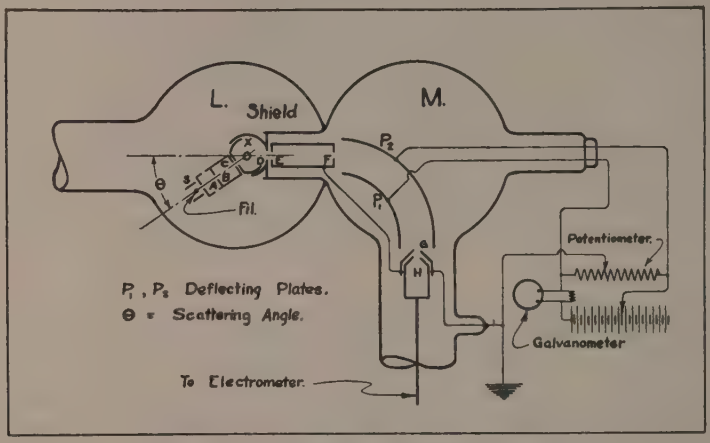

FIG. 1. *Diagram of the apparatus used.*

Dymond and Watson (3) used a much simpler electron gun which consisted of a filament and slit, the straight wire filament and the slit producing a narrow beam of electrons. In the experiments of Dymond and Watson (3), Hughes and McMillan (8), Harnwell (6) and those of the author, the electron gun could be rotated about an axis (O Fig. 1) at right angles to the line of the slits so that the angle of scattering could be varied. The results of the experiments on scattering will be given in a later section.

The analyser

The best known method of velocity analysis of beams of charged particles is to deflect the path of the particle in a magnetic field. One of the disadvantages of such a method is that it is very difficult to balance out the magnetic field in the small space where the collisions take place, a refinement which is necessary if one wishes to study the interaction of electrons and atoms in a state uninfluenced by external fields.

The electric method of analysis is to pass the beam of electrons between two curved plates. The potentials of the plates, relative to each other and relative to the slits, which are required to make the path of a beam of electrons of known velocity a circle, can be calculated from elementary electrostatic principles, provided the beam enters the analyser at a tangent to the required circular paths. If the beam has considerable angular divergence the problem is more

complicated. Hughes and Rojansky (9) and Hughes and McMillan (7) have found, both theoretically and experimentally, that refocussing takes place when the path of the beam has been bent through an angle of about 127°.

In the analyser used by the author, which was constructed some time before the above mentioned experiments were published, refocussing was not attempted. The path of the electron beam was bent by an electric field between two molybdenum plates whose radii of curvature were about 2.5 and 3.8 cm. Exact circles or an exact measurement of the radii of curvature of the plates (P_1 and P_2, Fig. 1) was of no great importance in this case because, owing to contact potentials etc., it was necessary to calibrate the analyser for electron beams of known velocity. If refocussing were attempted more accurate circles and a more exact knowledge of the geometry of the analyser would be of greater importance. As it was, the calculated and measured potentials between the plates, which would bring a beam of given velocity into the Faraday cylinder, differed only by a small percentage.

The method of applying the potential to the plates was the same as that used by Hughes and McMillan. The theoretical analysis shows that the potentials between slits F and G (same potential) and the two plates P_1 and P_2 bear a constant ratio to each other independent of the velocity of the electrons collected. With this in view a potentiometer arrangement was used, as shown in Fig. 1, to apply the potentials to the plates. The current in the potentiometer was measured with a galvanometer and shunt. After a calibration, the position of the galvanometer spot on the scale gives a direct measure of the velocity of the electrons being collected.

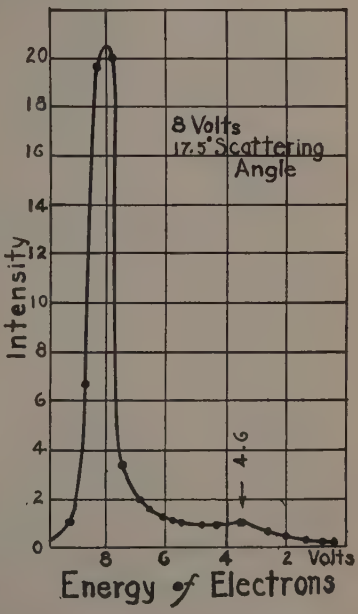

FIG. 2. *Energy losses in mercury for electrons having a primary energy of 8 volts.*

Resolving power

The curves discussed in the results will give the reader an idea of the resolving power of this analyser. The peaks were about a volt in width when 20-volt electrons were used, and could be located to about 0.2 volt. Peaks, one-third of a volt apart, could be detected. One of the interesting features of this analyser is the low voltages at which it could be used. The electron gun would not work at voltages below about six, but by retarding the electrons between slits D and E the analyser would work in a fairly satisfactory manner for electrons with energies as low as four volts. Of course great care had to be

used to balance out the earth's magnetic field, and the low voltage measurements were taken at night when disturbances due to local power lines would be at a minimum. The metal parts of the apparatus were all of copper and molybdenum and the whole apparatus could be baked, while evacuated, to a temperature of 300-400° C. Before baking, it was only with difficulty that the electron beam could be found in the analyser; after baking, however, even letting air into the apparatus and then re-exhausting had little effect on the position of the peaks.

The procedure was as follows: the potentials of slits A, B, C were so fixed as to obtain a good beam of electrons of the required velocity through slit C. For low velocities an accelerating potential of a few volts was usually maintained between slits D and E as it was more convenient to analyse the beam when it had about 20 volts energy. Then the current in the potentiometer was varied as required to bring electrons of different velocities into the Faraday cylinder. The electron current was measured with a Compton electrometer adjusted so that it gave about 5,000 divisions per volt. This would give a curve of the type shown in Fig. 2 to 6. Then the angle between the incident beam and the scattered beam was changed, and another velocity analysis taken.

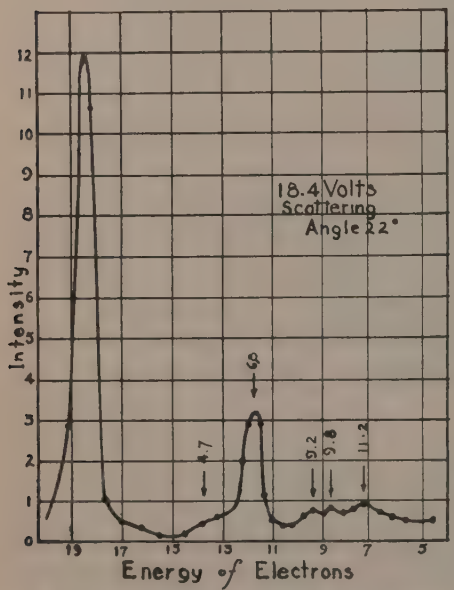

FIG. 3. *Energy losses in mercury for electrons having a primary energy of 18.4 volts.*

It is seen that this type of apparatus will give a measure of the energy losses in electron collisions with atoms as well as a measure of the angular distribution of the electrons which have lost different amounts of energy. Further advantages of the apparatus are that the space where the electrons are scattered can easily be kept field-free, (this cannot be done in the Ransauer type of apparatus used by Foard and by Whitney) and it will work at reasonably low voltages. It is hoped that this apparatus can be further perfected so that it can be used for much lower voltages still.

The apparatus was designed to be used for studying energy losses in gases as well as mercury vapor, and was used with circulating pumps to keep the analysing bulb M free from gas, while a reasonable pressure could be maintained in the bulb L. Two rapid mercury diffusion pumps, with a large low resistance liquid air trap, were connected to the bulb M by lengths of two-inch pyrex tubing as short as possible. The only connection between bulbs L and M was the slit D (0.3×2 mm.) which

was made in a piece of platinum foil, sealed to the tube inserted in bulb L. With the pumps running, a pressure ratio of 100 to 1 could be maintained between the two bulbs. As it was necessary to keep the analyser at a pressure of 10^{-4} mm. or less, the A side of the apparatus could be kept at a pressure as high as 0.01 mm.

Owing to lack of time no experiments were tried with gases except some preliminary runs with a mixture of mercury and helium. To use mercury vapor only, the liquid air trap on the A side of the apparatus was left at room temperature so that the pressure in L was that of mercury at room temperature. Unfortunately no more exact record of this pressure was kept than to see that it was steady over any particular run. For experiments on energy losses, the pressure did not matter much, but for the scattering experiments, more valuable results could have been obtained if the pressures had been known accurately.

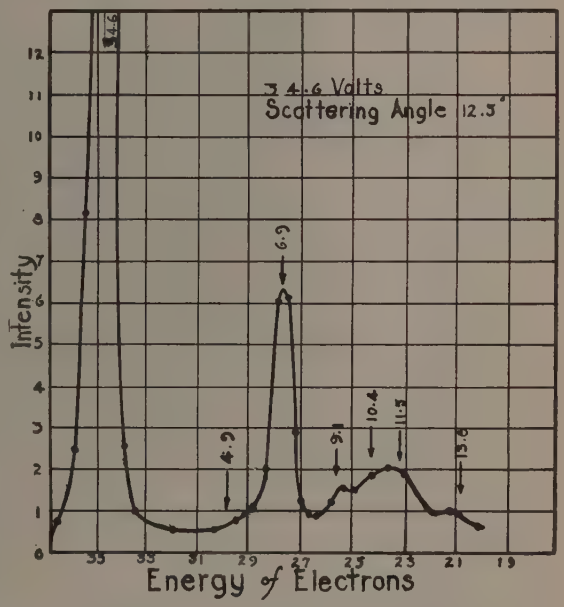

FIG. 4. *Energy losses in mercury for electrons having a primary energy of 34.6 volts.*

Results

Energy losses

Fig. 2-5 represent sample curves showing the energy losses of electrons of 8, 18.4, 34.6 and 49 volts respectively. These were the only voltages used. These curves were taken with the electron gun set so that the angle θ (Fig. 1) was 17.5, 22, 12.5 and 9.5 degrees respectively. The horizontal axis represents the energy of the electrons, and the vertical axis the number of electrons reaching the collector. The curves are not corrected for electrometer sensitivity, so the heights of peaks in different figures should not be compared.

Examining Fig. 5, for which the primary electron beam had an energy of 49 volts, the most intense peak on the left represents electrons which have been deflected without loss of energy. Then towards the right is a series of peaks which represent electrons that have lost definite amounts of energy on colliding with the mercury atoms. The position of these peaks in the voltage

scale gives a measure of the amount of energy lost. It is found that in most cases the amount of energy lost corresponds to one of the excitation potentials of mercury found spectroscopically.

The inelastic peaks may be identified as follows:

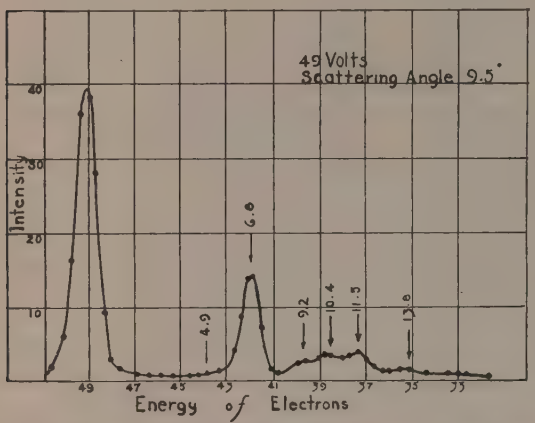

FIG. 5. *Energy losses in mercury for electrons having a primary energy of 49 volts.*

(1) An energy loss of about 4.9 volts. This peak probably includes the 4.7-volt, 4.9-volt, and 5.4-volt energy levels, corresponding to the 1^3P_0, 1^3P_1 and the 1^3P_2 levels. The resolving power was not sufficient to separate the 4.7-volt and 4.9-volt levels, and the 5.4-volt peak is too close to the 6.7-volt peak.

(2) An energy loss of 6.7 volts, the 1^1P_1 energy level. This peak is several times as intense as any other at all voltages studied except at eight volts, where the 4.9-volt peak appeared a little more prominent.

(3) An energy loss of about 9.2 volts. This probably corresponds to some of the many energy levels near ionization.

(4) An energy loss of 10.4 volts. This corresponds to ionization of the mercury atom.

(5) An energy loss of 11.5 volts. This may be the result of two successive collisions, one losing 4.7 volts or 4.9 volts, and the other 6.7 volts (see below).

(6) Another peak occurs at 13.5 to 14 volts. It is not sharp enough to be located accurately. It probably represents two collisions, each resulting in the loss of 6.7 volts.

Before discussing these results some reference should be made to the experiments of Whitney (13) and Foard (5). Their method of experiment, which insofar as energy loss observations are concerned has, up to the present, given more results than that used by the author, is different in that it gives no measure of the angle of scattering, and the collisions do not take place in a field-free space. Their method was somewhat similar to that used by many experimenters in studying the Ramsauer effect which need but little explanation here. The path of the electrons was curved immediately from the filament to the collector. The vapor was located in cylinders around the filament which separated it from the rest of the apparatus. Slits in the cylinders allowed the electrons to pass into the analysing space. The first two peaks were also found by Whitney to occur with about the same relative intensity. He worked at 16 different voltages between 5 and 41, and found a peak at 9.7 volts which probably corresponds to that found by the author at 9.2 He suggested that

this peak may be due to two successive collisions each of 4.9 volts loss. Whitney also obtained a peak at 11.7 volts which without doubt corresponds to that found in the present experiments at 11.5. The exact position of this peak is a little uncertain in both experiments; in Whitney's experiments it seemed to be mixed with, and inseparable from the ionization peak. No doubt, this is due to the fact that while he worked at a pressure corresponding to a temperature of 94° C., the pressure in the present experiments was that corresponding to about 25° C.

The work of Foard gave better results than that of Whitney, as the method was improved. He found the same peaks found by the author and some others besides. The resolving power was sufficiently high to separate the peaks corresponding to the 4.9 and 5.4-volt energy levels. His 4.9-volt peak probably includes the metastable 4.7-volt energy level as well, for there seems to be no reason why the 5.4-volt peak should occur and not that representing a loss of 4.7 volts or the 1^3P_0 level. The peak next in order is the 6.7-volt peak which is the most intense of all. It corresponds to the spectral line λ 1849 Å.

These energy loss observations, when they are put on a more quantitative basis, should be of great value in estimating the probabilities of excitation to the various energy levels many of which are difficult to observe spectroscopically, it is also important to compare these probabilities with those of excitation to a metastable state, a transition which cannot be observed spectroscopically.

Foard also found peaks corresponding to the 7.7 and 7.9-volt energy losses or to the 2^3S_1 and 2^1S_0 levels which were not found by Whitney nor in the course of the present work. His explanation of the fact that these were not found previously seems reasonable; he attributes it to the probability of excitation to these levels being very small except at voltages very close to the exciting potential. Between these peak voltages and the ionization voltage (10.4) Foard found two definite peaks at 8.8 and 9.8 volts which are easily connected with energy levels in the mercury atom; it is interesting to note, however, that neither Foard nor Whitney found any definite peaks at ionization while on the curves accompanying this paper a definite peak nearly always occurs at 10.4 volts or close enough thereto to be within experimental error. This discrepancy in observations in this neighborhood is perhaps due to a difference in pressure, and also to the fact that in Foard's apparatus the collisions can occur at any time during the acceleration of the electron to its final velocity, while in the present apparatus the collisions occur in a field-free space.

Losses above ionization

The energy losses above ionization found by Foard and the author are easily explicable except the 11.5-volt loss which no doubt corresponds to the two peaks found by Foard at 11.07 and 11.53. Even if an inaccuracy in measurement would allow an interpretation of this peak as being a 4.7+6.7 or 4.9+6.7-volt loss, its intensity would yet remain unexplained. In both the experiment

of Foard and the author's, and in spite of the large background* in his experiment, the loss is obviously much more intense than the 4.9-volt or the double 6.7-volt loss. This would be impossible if it were to be explained as a double collision. Further investigations of this type of energy loss probably will lead to information about the spectrum of mercury resulting from double transitions as suggested by Foard.

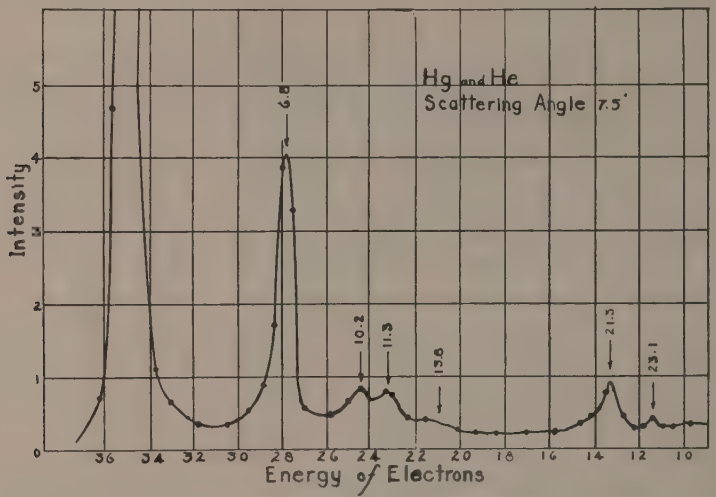

FIG. 6. *Energy losses in mercury-helium mixtures (primary energy 34.6 volts).*

Mixtures of helium and mercury

Some runs were taken with a mixture of helium and mercury vapor in the apparatus. This was due to an attempt to take some readings on helium without first thoroughly baking the mercury out of the apparatus. Fig. 6 shows a curve with helium at a pressure of 0.06 mm. The mercury energy loss peaks appear as before, and two peaks for helium appear at 21.3 and 23.1 volts which, without doubt, are the excitation and ionization peaks found by Dymond and Watson (3).

Scattering

At each of the four voltages an analysis of the energy losses was taken at different scattering angles between 0 and about 60°. A scattering curve for each type of peak can be obtained by finding the area of the peak, omitting the background where it is appreciable, correcting for the fact that the effective volume of the part in which the scattering takes place is different at different angles (multiplying the intensity by sin θ) and plotting against the angular position of the electron gun. The curves representing the angular distribution

* *A considerable number of electrons, having a continuous velocity spectrum, make intensity comparisons among the peaks difficult. These form a "background" to the curve, representing the velocity analysis.*

of electrons scattered by elastic collisions at the four voltages studies are shown in Fig. 7. These curves are not very accurate because of some uncertainty due to the background scattering. Foard found a great deal of such scattering, particularly on the part of the velocity curve where the energy loss approached the energy of the incident beam. Without doubt his was mainly instrumental, though in his curves as well as in the present ones it seems more pronounced near ionization. No doubt this is due to the fact that the energy loss is not necessarily quantized at ionization. As the slits defined a beam about 3° wide, the slit scattering should be negligible for angles greater than about 10° and could hardly affect the result at large angles. However, at large angles the accuracy is also reduced by lack of intensity. It is noted that in general the higher the voltage the less steep the scattering curve. This is in general agreement with a curve shown by Arnot (1) for elastically scattered electrons of 82 volts energy. He used retarding potentials to separate the elastic and inelastic beams. If his curve is fitted on those in Fig. 7 it is still less steep than the curve marked 49 volts. The dotted line represents an inverse square law type of scattering where the number of electrons scattered through a definite angle varies as $\operatorname{cosec}^4 \frac{\theta}{2}$.

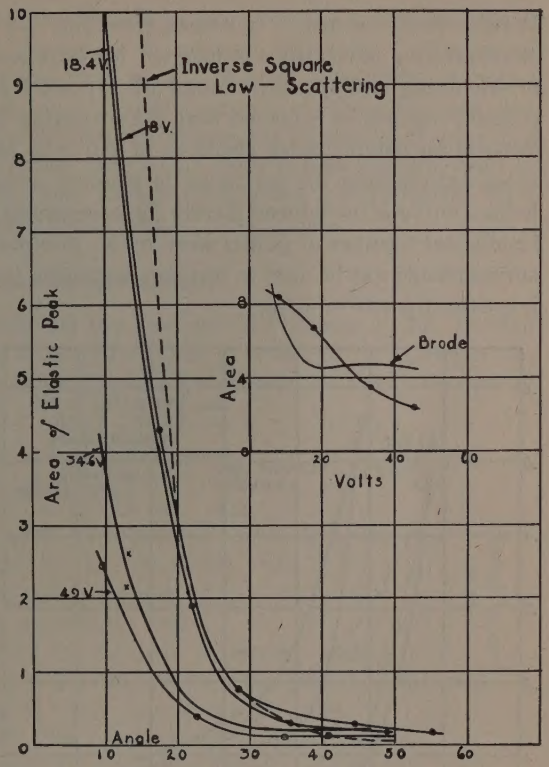

FIG. 7. *Angular distribution of elastically scattered electrons.*

Fig. 8 shows the scattering curve for the electrons having lost 6.7 volts, at initial voltages of 34.6 and 18.4 volts. Curves for the other voltages are not present because at eight volts the 6.7-volt loss does not appear. The measurements on the 6.7-volt loss peak, for collisions at 49 volts were not satisfactory; they indicate a curve which has even a steeper slope than that at 34.6 volts, though it is like it in other respects. The broken line curves in Fig. 8 represent the scattering curves for the elastic peaks, or the same curves shown in Fig. 7 drawn to a reduced scale so that they fit the inelastic scattering curve at one point. A curve for elastic scattering in helium copied from Dymond and

Watson is also shown. It is seen from Fig. 7-8 that the curve representing the scattering of electrons in mercury lies between the inverse square law type of scattering and the type found by Dymond and Watson for helium. Of course it cannot be expected that the scattering curve found by Dymond and Watson for helium, using electrons of 210 volts energy, should be the same as those representing the scattering in mercury at much lower voltages, but the helium curve is introduced merely for comparison. Owing to the fact that an insufficient number of points were taken to obtain any great accuracy, these curves should not be used to test any scattering theory unless the disagreement is greater than 20 or 30%.

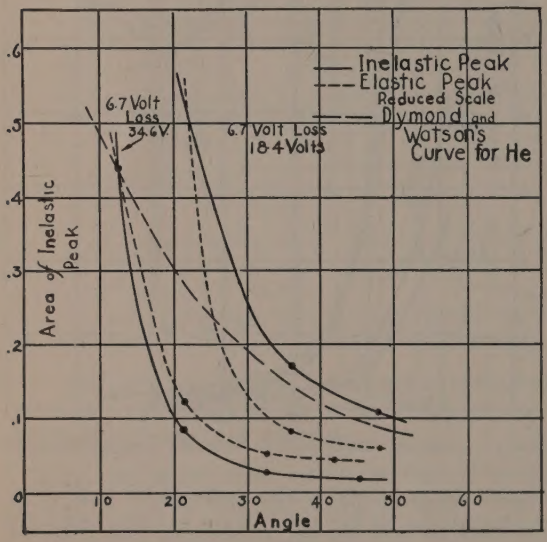

FIG. 8. *Angular distribution of inelastically scattered electrons.*

It will be noticed that at 18.4 volts (Fig. 8) the intensity of the 6.7-volt inelastic peak relative to the corresponding elastic peak is different from the curves taken at 34.6 volts and at 49 volts. (The 49-volt curve seemed to be the same as the 34.6-volt curve in this respect.) At the higher voltages the relative intensity of the elastic to inelastic peak was greater at small than at large angles. At 18.4 volts the inverse seems to be true. There is no reason to suppose that this is due to any instrumental error but the point should be reinvestigated. It was definitely noticeable that the intensity of the inelastic peak was always much greater relatively at this voltage than at the higher voltages studied, as can be seen from the curves in Fig. 8. The curve taken by Brode (2) representing the coefficient of absorption of slow electrons in mercury has a minimum at about this voltage. The intensities of the inelastic peaks representing greater energy losses were too low to give a scattering curve worth consideration.

The total amount of scattering greater than a given angle should give a measure of the effective target area of a mercury atom to electrons at the different voltages. The area of the curves in Fig. 7 for angles between 20 and 50° is shown in the inset in Fig. 7. This is compared with Brode's curve for the coefficient of absorption of electrons in mercury vapor and little agreement is found. Of course, to get an accurate comparison, all angles greater than

2 or 3° should be included and the inelastic peaks as well as the elastic. However, the direction of the change is in general the same. This curve representing the total scattering has even a higher probability of error than those in Fig. 7 because, as previously mentioned, the apparatus was subject to temperature changes which would change the pressure of the mercury vapor slightly. It would not affect any of the other curves but that inset in Fig. 7, as the readings at any one voltage from which all the curves were plotted were taken under steady conditions.

Acknowledgment

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